§ 620.46 General requirements.

(a) *Dose.* These standards are based on (1) vaccine intended for intradermal injection in a single human immunizing dose of 0.1 milliliter and (2) vaccine intended for percutaneous injection in a single skin application through which inoculation is made by a multiple puncture device.

(b) *Date of manufacture.* The date of manufacture is the date of initiation of the last valid determination for CFU after freeze-drying.

§ 620.47 Labeling.

In addition to conforming to the applicable requirements of §§ 610.60, 610.61, and 610.62 of this chapter, the package label must bear the following information:

(a) Specification of the route of administration.

(b) A statement that the vaccine contains live bacteria and should be protected against exposure to light.

(c) A statement that the vaccine must be administered within 8 hours after reconstitution, and that reconstituted vaccine not used within 8 hours must be discarded.

§ 620.48 Samples; protocols; official release.

(a) For each lot of vaccine, the following materials must be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.

(1) Samples and diluent that will provide at least 20 milliliters when the samples are reconstituted as recommended in the package insert by the manufacturer of the vaccine.

(2) A protocol that consists of a complete summary of the manufacture of each lot, including all results of each test required by all applicable regulations. If the protocol is not included in the shipment of the samples, it must be sent promptly to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.

(b) The BCG Vaccine must not be issued by the manufacturer until written notification of official release is received from the Director, Center for Biologics Evaluation and Research, Food and Drug Administration.

[44 FR 14545, Mar. 13, 1979, as amended at 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

PART 630—ADDITIONAL STANDARDS FOR VIRAL VACCINES

Subpart A—Poliovirus Vaccine Inactivated

Subpart B—Poliovirus Vaccine Live Oral Trivalent

Subpart C—[Reserved]

Subpart D—Measles Virus Vaccine Live

Subpart E—[Reserved]

Subpart F—Mumps Virus Vaccine Live

Subpart G—Rubella Virus Vaccine Live

Subpart H—Smallpox Vaccine

AUTHORITY: Secs. 201, 501, 502, 503, 505, 510, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 351, 352, 353, 355, 360, 371); secs. 215, 351, 352, 353, 361 of the Public Health Service Act (42 U.S.C. 216, 262, 263, 263a, 264).

SOURCE: 38 FR 32068, Nov. 20, 1973, unless otherwise noted.

CROSS REFERENCES: For U.S. Customs Service regulations relating to viruses, serums, and toxins, see 19 CFR 12.21—12.23. For U.S. Postal Service regulations relating to the admissibility to the United States mails see parts 124 and 125 of the Domestic Mail Manual, that is incorporated by reference in 39 CFR part 111.

Subpart A—Poliovirus Vaccine Inactivated

§ 630.1 Poliovirus Vaccine Inactivated.

(a) *Proper name and definition.* The proper name of this product shall be ''Poliovirus Vaccine Inactivated'' which shall consist of an aqueous preparation of poliovirus types 1, 2, and 3, grown in monkey kidney tissue cultures, inactivated by a suitable method.

(b) *Strains of virus.* Strains of poliovirus used in the manufacture of vaccine shall be identified by historical records, infectivity tests and immunological methods. Any strain of virus may be used that produces a vaccine meeting the requirements of §§ 630.2, 630.3, and 630.4, but the Director, Center for Biologics Evaluation and Research may from time to time prohibit the use of any specific strain whenever he finds that it is practicable to use another strain of the same type that is potentially less pathogenic to man and that will produce a vaccine of at least equivalent safety and potency.

(c) *Monkeys; species permissible as source of kidney tissue.* Only Macaca or Cercopithecus monkeys, or a species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable, which have met all requirements of §§ 600.11(f)(2) and 600.11(f)(8) of this chapter shall be used as a source of kidney tissue for the manufacture of Poliovirus Vaccine Inactivated.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 50 FR 4137, Jan. 29, 1985; 55 FR 11013, Mar. 26, 1990]

§ 630.2 Poliovirus Vaccine Inactivated.

(a) *Cultivation of virus.* Virus for manufacturing vaccine shall be grown with aseptic techniques in monkey kidney cell cultures. Suitable antibiotics in the minimum concentration required may be used (§ 610.15(c) of this chapter).

(b) *Filtration.* Within 72 hours preceding the beginning of inactivation, the virus suspensions shall be filtered or clarified by a method having an efficiency equivalent to that of filtration through an S1 Seitz type filter pad.

(c) *Virus titer.* The 50 percent endpoint ($TCID_{50}$) of the virus fluids after filtration shall be $10^{6.5}$ or greater as confirmed by comparison in a simultaneous test (using groups of 10 tubes at 1 log steps or groups of 5 tubes at 0.5 log steps) with a reference virus distributed by the Center for Biologics Evaluation and Research. Acceptable titrations of the reference virus shall not vary more than ± 0.5 $\log_{10}$ from its labeled titer using 0.5 milliliter inoculum in tissue culture.

(d) *Inactivation of virus.* The virus shall be inactivated, as evidenced by the tests described in § 630.4, through the use of an agent or method which has been demonstrated to be consistently effective in the hands of the manufacturer in inactivating a series of lots of poliovirus. If formaldehyde is used for inactivation, it shall be added to the virus suspension to a final concentration of U.S.P. solution of formaldehyde of 1:4000, and the inactivation conducted under controlled conditions of pH and time, at a temperature of 36° to 38° C. Three or more virus titers, suitably spaced to indicate rate of inactivation, shall be determined during the inactivation process. Filtration equivalent to that described in paragraph (b) of this section shall be performed after the estimated baseline time (time at which the 50 percent end-

point reaches one tissue culture infective dose per milliliter), but prior to sampling for the first single strain tissue culture test required in §630.4(b), except that this filtration may be omitted for strains of a virulence for monkeys equal to or less than that of the MEF–1 Type 2 strain of poliovirus.

(e) *Additional processing.* Single strain or trivalent pools that have failed to pass safety tests prescribed in §630.4 (b), (c), or (e) may be treated as follows:

(1) Filtration or clarification by a method having an efficiency equivalent to that of filtration through an S1 Seitz type filter pad.

(2) Negative tests performed as described in §630.4 (b) and (c) must be obtained on each of two successive samples taken so as to be separated by an interval of at least 3 days while the material is being subjected to treatment with 1:4000 U.S.P. formaldehyde solution and heat at 36° to 38° C. The first sample may be taken before incubation is begun and the second sample shall be taken after the incubation of at least 3 days is completed. For both single strain and trivalent pools the volume tested for each tissue culture safety test shall be equivalent to at least 1,500 human doses.

(3) Pools which are positive following such additional processing shall not be used for the manufacture of Poliovirus Vaccine Inactivated

(f) *Supplemental inactivation.* Supplemental inactivation employing a method capable of reducing the titer of a similarly produced virus suspension by a factor of 10^{-6} may be applied at any point after the filtration step described in paragraph (d) or (e)(1) of this section.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 50 FR 4137, Jan. 29, 1985; 55 FR 11013, Mar. 26, 1990]

§630.3 Potency test.

Each lot of vaccine shall be subjected to a potency test which permits an estimation of the antigenic capacity of the vaccine. This is done by means of a simultaneous comparison of the serum antibody levels produced in monkeys by the vaccine under test with the antibody level of the reference serum distributed by the Center for Biologics Evaluation and Research. The potency test shall be performed on samples taken after all final processing of the product has been completed, including addition of preservative, except that when the final product contains material having an adjuvant effect an additional test shall be performed with a sample taken before the addition of the adjuvant material. The volume of the test sample for the additional test shall be adjusted to the equivalent volume of Poliovirus Vaccine Inactivated in the final product. The test shall be conducted as follows:

(a) *Inoculation of monkeys.* A group of 12 or more Macaca monkeys, or a species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable for the purpose, shall be used. Animals shall weigh between 4 and 8 pounds and shall be in overt good health. Animals that become ill and remain ill during the course of immunization shall be excluded from the group. The test shall not be valid unless at least 10 animals survive the test period and their preinoculation serum antibody levels are as prescribed in paragraph (d) of this section. The test vaccine shall be given intramuscularly to each monkey in 3 doses at 7-day intervals, each dose to be the recommended individual human dose. Only undiluted vaccine shall be used.

(b) *Serum samples.* A blood sample shall be taken from each monkey prior to vaccination and then again 7 days after the last injection. Serum shall be separated aseptically, and stored under refrigeration.

(c) *Serum-virus neutralization test.* The titers of individual monkey serums shall be determined in comparison with the reference serum in tests designed to include controls for all the variables of significance including the following:

(1) Serum toxicity control;

(2) Cell control and cell titration;

(3) Virus titration control (at least 4 tubes for each dilution at 0.5 log steps); and

(4) Serum controls using type-specific serums to identify the type of virus used in the neutralization test.

(d) *Interpretation of the test.* Animals showing preinoculation titers of 1:4 or over when tested against not more

than 1,000 $TCID_{50}$ of virus, shall be excluded from the test. The geometric mean titer of antibody induced in the monkeys surviving the course of immunization and bleeding, shall be calculated. A comparison of the value so obtained shall be made with the value for the reference serum that was tested simultaneously and expressed as the ratio between the geometric mean titer value of the serums under test and the mean titer value of the reference serum.

(e) *Potency requirements.* A lot of vaccine tested against the reference serum shall be satisfactory if the geometric mean value of the group of individual monkey serums representing the lot of vaccine tested is at least 1.29 times the mean value of the reference serum for Type 1, at least 1.13 times for Type 2, and at least 0.72 times for Type 3.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 50 FR 4137, Jan. 29, 1985; 55 FR 11013, Mar. 26, 1990]

§ 630.4 Tests for safety.

In the manufacture of the product, the following tests relating to safety shall be conducted by the manufacturer.

(a) *The virus pool—tests prior to inactivation*—(1) *B virus and Mycobacterium tuberculosis.* Prior to inactivation, each individual virus harvest or virus pool shall be tested for the presence of B virus and Mycobacterium tuberculosis.

(2) *SV–40.* Prior to inactivation, the material shall be tested for the presence of SV–40 as follows (or by any other test producing equally reliable results): A sample of at least 5 ml. from the virus harvest or virus pool shall be neutralized by high titer specific antiserum of other than primate origin. A similar sample from the pool of tissue culture fluids from control vessels representing the tissue from which the virus was prepared may be tested in place of the virus sample. The sample shall be tested in primary cercopithecus tissue cultures or in a cell line demonstrated as at least equally susceptible to SV–40. Each tissue culture system shall be observed for at least 14 days and at the end of the observation period at least one subculture of fluid shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days.

(3) *Test results.* The virus harvest or virus pool is satisfactory for poliovirus vaccine only if the tests produce no evidence of the presence of B virus, Mycobacterium tuberculosis or SV–40.

(b) *Single strain pool tissue culture tests for poliovirus.* (1) Before pooling to make the final poliovirus vaccine, during inactivation at 36° to 38° C., two samples of each monovalent bulk strain pool shall be tested for the presence of virus by tissue culture methods, the second sample to be taken at least 3 days after taking the first sample.

(2) Each sample shall be no smaller than the equivalent of 1,500 human doses and shall be subjected to the complete testing process and each test shall be performed on a different monkey kidney tissue culture cell preparation. The test sample for one of these tests may be used also for the test prescribed in paragraph (f) of this section provided the cell cultures used have been demonstrated as fully susceptible to SV–40 and poliovirus. Each sample shall be inoculated into five or more tissue culture bottles of a suitable capacity, the ratio of the vaccine to the nutrient fluid being approximately 1:1 to 1:3, and the area of the surface growth of cells being at least 3 square centimeters per milliliter of sample. The tissue culture bottles shall be observed for at least 14 days.

(3) A first subculture shall be made at the end of 7 days from date of inoculation by planting at least 2 percent of the volume from each original bottle into suitable tissue culture vessels, followed by refeeding.

(4) A second subculture shall be made from each original bottle in the same manner at the end of 14 days from date of inoculation.

(5) Each of the first and second subcultures shall be observed for at least 7 days.

(6) If cytopathogenic effects occur either in the original bottles of the two tests or in the subcultures from them, or if cellular degeneration appears in the original bottles or in the subcultures before degeneration occurs in uninoculated cultures, the pool shall be

held until the matter is resolved. If active poliovirus is indicated, the strain pool shall not be used for inclusion in a final vaccine unless effectively reprocessed as described in §630.2(e). If other viruses are present, the pool shall not be used unless it can be demonstrated that such viruses have originated from other than the strain pool being tested.

(c) *Trivalent vaccine pool tissue culture test.* No less than 1,500 human doses of the trivalent vaccine pool, without final preservative, prepared by pooling the three type pools, each of which has passed all tests prescribed in paragraph (b) of this section, shall be subjected to the complete tissue culture test prescribed in such paragraph (b) in at least two approximately equal tests in separate monkey kidney tissue culture preparations. This test sample may be used also for the test prescribed in paragraph (f) of this section provided the cell cultures used have been demonstrated as fully susceptible to SV–40 and poliovirus.

(d) *Trivalent vaccine pool lymphocytic choriomeningitis test.* The final vaccine shall be shown to be free of lymphocytic choriomeningitis virus by intracerebral inoculation of the maximum volume tolerated into 10 or more mice which shall be observed daily for at least 21 days and a negative test shall not be valid unless at least eight mice survive for this period.

(e) *Test in monkeys for active virus.* (1) Vaccine from final containers selected at random from each filling of each lot shall be pooled to provide a test sample of at least 400 milliliters representing the various fillings. An equal volume of bulk vaccine may be substituted for test samples from each filling lot provided the procedure has been approved by the Director, Center for Biologics Evaluation and Research.

(2) A total of not less than 20 monkeys shall be inoculated with the test sample. A preinjection serum sample from each monkey must not contain neutralizing antibody against the three poliovirus types detectable in a dilution of 1:4 when tested against not more than 1,000 $TCID_{50}$ of virus. At least 80 percent of the test animals representing each filling or each bulk sample must survive the test period without significant weight loss, except that if at least 60 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48-hour test period may be replaced by an equal number of test animals. At least 80 percent of the animals used in the test must show microscopic evidence of inoculation trauma in the lumbar region of the spinal cord, and gross or microscopic evidence of inoculation trauma in the thalamic area. If less than 60 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals fail to meet the other criteria prescribed in this section, the test must be repeated.

(3) Vaccines shall be injected by combined intracerebral, intraspinal, and intramuscular routes into Macaca or Cercopithecus monkeys or a species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable for the purpose. The animals shall be in overt good health and injected under deep barbiturate anesthesia. The intracerebral injection shall consist of 0.5 milliliter of test sample into the thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 milliliter of concentrated test sample into the lumbar spinal cord enlargement, the test sample to be concentrated 100 fold in the ultracentrifuge by a method demonstrated to recover at least 90 percent of the virus particles in the sediment after it has been resuspended in the same lot of unconcentrated test sample. The intramuscular injection shall consist of 1.0 milliliter of test sample into the right leg muscles. At the same time, 200 milligrams of cortisone acetate shall be injected into the left leg muscles, and 1.0 milliliter of procaine penicillin (300,000 units) into the right arm muscles. The monkeys shall be observed for 17 to 19 days and signs suggestive of poliomyelitis shall be recorded.

(4) At the end of the observation period, samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identification. Histological sections shall be prepared from both spinal cord enlargements and examined.

(5) Doubtful histopathological findings necessitate (i) examination of a sample of sections from several regions of the brain in question, and (ii) attempts at virus recovery from the nervous tissues previously removed from the animal. The test results must be negative. Test results are negative if the histological and other studies leave no doubt that poliovirus infection did not occur.

(f) *Tissue culture safety test for SV–40.* At least 500 human doses of each monovalent or trivalent pool of vaccine shall be tested for the presence of SV–40 using primary cercopithecus monkey tissue cultures or using a cell line demonstrated as at least equally susceptible to SV–40. The test shall be conducted as described in paragraph (b) of this section, except for the volume of test sample and except that one subculture of at least 2 percent of the volume of the fluids shall be made no less than 14 days from the date of inoculation and examined for at least 14 days from the date of subinoculation. The vaccine is satisfactory only if there is no evidence of the presence of SV–40 in any of the cultures or subcultures.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 50 FR 4137, Jan. 29, 1985; 50 FR 16229, Apr. 25, 1985; 55 FR 11013, Mar. 26, 1990; 57 FR 10814, Mar. 31, 1992]

§ 630.5 General requirements.

(a) *Consistency of manufacture.* No lot of final vaccine shall be released unless it is one of a series of five consecutive lots produced by the same manufacturing process, all of which have shown negative results with respect to all tests for the presence of live poliovirus, and unless each of the monovalent pools of which a polyvalent final vaccine is composed similarly is one of a series of five consecutive monovalent pools of the same type of inactivated poliovirus, all of which have shown negative results in all tests for the presence of live poliovirus.

(b) *Dose.* These additional standards are based on a human dose of 1.0 milliliter for a single injection and a total human immunizing dose of three injections of 1.0 milliliter given at appropriate intervals.

(c) *Samples and protocols.* For each lot of vaccine, the following material shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892:

(1) A 2,500 milliliter sample, neutralized, not dialyzed, and without final preservative, taken at the latest possible stage of manufacturing before the addition of such preservative.

(2) A 200 milliliter bulk sample of the final vaccine containing final preservative.

(3) A total of not less than a 200 milliliter sample of the final vaccine in final labeled containers.

(4) A protocol which consists of a summary of the history of manufacture of each lot including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 51 FR 18580, May 21, 1986; 55 FR 11013, Mar. 26, 1990]

Subpart B—Poliovirus Vaccine Live Oral Trivalent

SOURCE: 56 FR 21432, May 8, 1991, unless otherwise noted.

§ 630.10 Poliovirus Vaccine Live Oral Trivalent.

(a) *Proper name and definition.* The proper name of this product shall be Poliovirus Vaccine Live Oral Trivalent. The vaccine shall be a preparation containing the three types of live, attenuated polioviruses grown in monkey kidney cell cultures, or in a cell line found by the Director, Center for Biologics Evaluation and Research, to meet the requirements of §610.18(c) of this chapter. The vaccine shall be prepared in a form suitable for oral administration.

(b) *Criteria for acceptable strains.* (1) The Sabin strains of attenuated poliovirus, Type 1 (LS-c, 2ab/KP_2), Type 2 (P712, Ch, 2ab/KP_2), and Type 3 (Leon $12a_1b/KP_3$), or derivatives from them, may be used in the manufacture of vaccine.

(2)(i) Other poliovirus strains may be used in the manufacture of Poliovirus Vaccine Live Oral Trivalent provided that they are identified by historical records including:

(A) Origin,

(B) Techniques of attenuation,

(C) Antigenic properties,

(D) Neurovirulence for monkeys,

(E) Pathogenicity for tissue cultures of various cell types, and

(F) Established virus markers, including rct/40, and d.

(ii) The data shall be submitted to the Director, Center for Biologics Evaluation and Research, along with other data that establish:

(A) That each such strain is at least as safe as the Sabin strain of the corresponding type,

(B) That each such strain demonstrates results comparable to the Sabin srain when inoculated into monkeys by the intrathalamic and intramuscular routes, and

(C) That each such strain has been used to produce vaccines meeting the safety and potency requirements of §§ 630.11, 630.15, 630.16 or 630.17, and 630.18.

(3) The Director, Center for Biologics Evaluation and Research, may prohibit the use of a specified strain whenever the Director finds that it is practicable to use another strain of the same type that will produce a vaccine of greater safety and of at least equivalent potency.

(4) If vaccine lots have been produced directly from strain materials (e.g., Sabin Original, Sabin Original Merck, or Sabin Original Rederived), the strain material is not required to be tested in accordance with the provisions of § 630.10(c).

(c) *Criteria for qualification of the seed virus.* (1) Each seed virus used in vaccine manufacture shall be prepared from an acceptable strain in monkey kidney cell cultures, derived from animals which have met all of the requirements of § 630.12(a), or in a cell culture of a type determined to be suitable by the Director, Center for Biologics Evaluation and Research. The seed virus used in vaccine manufactures shall be demonstrated to be free of extraneous microbial agents except for unavoidable bacteriophage.

(2) Seed virus used for the manufacture of oral poliovirus vaccine shall meet the requirements of §§ 630.13, 630.16 or 630.17, and 630.18. In addition, the neurovirulence of each of the first five consecutive monovalent virus pools prepared from the seed virus shall meet the neurovirulence requirements prescribed in §§ 630.16(b)(2) or 630.17 (b)(3).

(3) A new seed virus may be used for production provided data are submitted in the form of a product license a supplement that show the new seed virus and each of the first five consecutive monovalent virus pools prepared from it meet the safety requirements of §§ 630.13 and 630.16 or 630.17 and 630.18 and approval for the use of the seed virus is received in writing from the Director, Center for Biologics Evaluation and Research.

(4) Seed virus in vaccine manufacture shall be prepared in a seed lot system from a master virus seed lot at a passage level consistent with § 630.13(a).

(5) For monovalent virus pools tested in accordance with § 630.16(b), the use of the seed virus may continue provided that the frequency of monovalent virus pools produced with it which fail to meet the criteria of neurovirulence for monkeys prescribed in § 630.16(b)(2) is not greater than predicted on the basis of comparison with the corresponding reference preparation. If the frequency of monovalent virus pools produced with the same seed virus which fail to meet the criteria of neurovirulence for monkeys prescribed in §§ 630.16(b)(2) is greater than the predicted 1 percent on the basis of the 99-percent fiduciary one-sided upper limit, that seed virus shall be disqualified for further use in vaccine production.

(6) For monovalent virus pools tested in accordance with § 630.17, subsequent and identical neurovirulence tests of the seed virus shall be performed in monkeys whenever there is evidence of a significant increase in the neurovirulence of the seed virus, upon introduction of a new production seed lot, and as often as is necessary to otherwise establish, to the satisfaction of the Director, Center for Biologics Evaluation and Research, that the seed virus for vaccine manufacture has maintained its neurovirulence properties as set forth in § 630.17 (b)(3).

[56 FR 21432, May 8, 1991, as amended at 59 FR 49351, Sept. 28, 1994]

§ 630.11 Clinical trials to qualify for license.

To qualify for license, the antigenicity of the vaccine shall have been determined by clinical trials of adequate statistical design conducted in compliance with part 56 of this chapter, unless exempted under § 56.104 or granted a waiver under § 56.105, and with part 50 of this chapter. Such clinical trials shall be conducted with five lots of oral poliovirus vaccine that have been manufactured by the same methods. Type specific neutralizing antibody for each type of poliovirus in the vaccine shall be induced in 90 percent or more of susceptibles after a series of doses.

§ 630.12 Animal source and quarantine; personnel.

(a) *Monkeys*—(1) *Species permissible as source of kidney tissue.* Only Macaca monkeys, Cercopithecus monkeys, or other species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable, which meet the requirements of § 600.11 (f)(2) and (f)(8) of this chapter, shall be used as the source of kidney tissue for the manufacture of Poliovirus Vaccine Live Oral Trivalent.

(2) *Experimental and test monkeys.* Monkeys that have been used previously for experimental or test purposes shall not be used as a source of kidney tissue in the processing of vaccine.

(3) *Quarantine; additional requirements.* Excluding deaths from accidents or causes not due to infectious diseases, if the death rate of any group of monkeys being conditioned in accordance with § 600.11(f)(2) of this chapter exceeds 5 percent per month, the remaining monkeys may be used for the manufacture of Poliovirus Vaccine Live Oral Trivalent only if all of the monkeys survive a new quarantine period.

(b) *Personnel.* All reasonably possible steps shall be taken to ensure that personnel involved in processing the vaccine are immune to all three types of poliovirus and do not excrete poliovirus.

[56 FR 21432, May 8, 1991; 56 FR 27787, June 17, 1991]

§ 630.13 Manufacture of Poliovirus Vaccine Live Oral Trivalent.

(a) *Virus passages.* Virus in the final vaccine shall represent no more than five tissue culture passages from the original strain or no more than five tissue culture passages from a virus clone derived from one of the first five tissue culture passages of the original strain.

(b) *Virus propagated in primary monkey kidney cell cultures*—(1) *Continuous cell lines.* When primary monkeys kidney cell cultures are used in the manufacture of poliovirus vaccine, continuous cell lines shall not be introduced or propagated in vaccine manufacturing areas.

(2) *Identification of processed kidneys.* The kidneys from each monkey shall be processed separately. The resulting viral fluid shall be identified as a separate monovalent harvest and kept separately from other monovalent harvests until all samples for the tests prescribed in paragraphs (b)(3) and (b)(4) of this section relating to that pair of kidneys have been withdrawn from the harvest.

(3) *Monkey kidney tissue production vessels prior to virus inoculation.* Prior to inoculation with the seed virus and at least 3 days after complete formation of the tissue sheet, the tissue culture growth in vessels derived from each pair of kidneys shall be examined microscopically for evidence of cell degeneration. If such evidence is observed, the tissue cultures from that pair of kidneys shall not be used for poliovirus vaccine manufacture. To test the tissue found free of cell degeneration for further evidence of freedom from demonstrable viable microbial agents, the fluid shall be removed from the cell cultures immediately prior to virus inoculation and tested in each of four culture systems:

(i) Macaca monkey kidney cells,

(ii) Cercopithecus monkey kidney cells,

(iii) Primary rabbit kidney cells, and

(iv) Cells from one of the systems described in § 630.18(a)(6).

The fluid shall be tested in the following manner: Aliquots of fluid from each vessel derived from the same pair of kidneys shall be pooled and at least 10 milliliters of the pool inoculated into each system. The dilution of the

pool with medium shall be no greater than 1:4 and the area of surface growth of cells shall be at least 3 square centimeters per milliliter of test inoculum. The cultures shall be observed for at least 14 days. At the end of the observation period, at least one subculture of fluid from the Cercopithecus monkey kidney cell cultures shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days. If these tests indicate the presence in the monkey kidney tissue culture production vessels of any viable microbial agent, the viral harvest from these tissue cultures so implicated shall not be used for poliovirus vaccine manufacture.

(4) *Control vessels.* At least 25 percent of the cell suspension from each pair of kidneys shall be set aside and used to establish control cultures. The control cultures shall be examined microscopically for cell degeneration for an additional 14 days. The culture fluids from such control cells shall be tested, both at the time of virus harvest and at the end of the additional observation period, by the method prescribed for testing of fluids in paragraph (b)(3) of this section. In addition, the control cell sheet shall be examined for presence of hemadsorbing viruses by the addition of guinea pig red blood cells.

(5) *Interpretation of test results.* At least 80 percent of the control vessels shall be free of cell degeneration at the end of the observation period to qualify the kidneys for poliovirus vaccine manufacture. If the test results of the control cells indicate the presence of any extraneous agent at the time of virus harvest, the virus harvest from that tissue culture preparation shall not be used for poliovirus vaccine manufacture. If any of the tests or observations described in paragraph (b)(3) or (b)(4) of this section demonstrate the presence in the tissue culture preparation of any microbial agent known to be capable of producing human disease, the virus grown in each tissue culture preparation shall not be used for poliovirus vaccine manufacture.

(6) *Temperature of kidney tissue production vessels after virus inoculation.* After virus inoculation, production vessels shall be maintained at 33.0 to 35.0 °C during the course of virus propagation.

(7) *Kidney tissue virus harvests.* Virus shall be harvested not later than 72 hours after virus inoculation. Virus harvested from vessels containing the kidney tissue from one monkey may be tested separately, or samples of viral harvests from more than one pair of kidneys may be combined, identified, and tested as a monovalent virus pool. Each pool shall be mixed thoroughly and samples withdrawn for testing as prescribed in §630.18(a). The samples shall be withdrawn immediately after harvesting and prior to further processing, except that samples of test materials frozen immediately after harvesting and maintained at −60 °C or below, may be tested upon thawing, provided no more than one freeze-thaw cycle is employed.

(8) *Filtration.* After harvesting and removal of samples for the safety tests prescribed in §630.18(a), the pool shall be passed through sterile filters having a sufficiently small porosity to assure bacteriologically sterile filtrates.

[56 FR 21432, May 8, 1991, as amended at 58 FR 19609, Apr. 15, 1993]

§630.14 Reference virus preparations.

(a) *Titration test controls.* The following reference viruses may be obtained from the Center for Biologics Evaluation and Research:

(1) Reference Poliovirus, Live, Attenuated, Type 1, as a control for correlation of virus titers in tissue cultures.

(2) Reference Poliovirus, Live, Attenuated, Type 2, as a control for correlation of virus titers in tissue cultures.

(3) Reference Poliovirus, Live, Attenuated, Type 3, as a control for correlation of virus titers in tissue cultures.

(4) Reference Poliovirus, Live, Attenuated, Trivalent, as a control for correlation of virus titers in tissue cultures.

(b) *Neurovirulence test controls.* (1) Except as provided in paragraph (b)(2) of this section, the following reference virus may be obtained from the Center for Biologics Evaluation and Research:

(i) Reference Attenuated Poliovirus, Type 1, as a control for evaluation of monkey neurovirulence tests.

(ii) Reference Attenuated Poliovirus, Type 2, as a control for evaluation of monkey neurovirulence tests.

(iii) Reference Attenuated Poliovirus, Type 3, as a control for evaluation of monkey neurovirulence tests.

(2) Alternatively, upon FDA approval, World Health Organization (WHO) reference standards of the corresponding type, WHO/I, WHO/II, and WHO/III, may be used as controls for evaluation of monkey neurovirulence tests.

§ 630.15 Potency test.

(a) *Test for virus titer.* The concentration of living virus in each monovalent virus pool and in each trivalent vaccine, expressed as infectivity titer per milliliter for cell cultures, shall be determined using the Reference Poliovirus, Live, Attenuated of the same type as a control or using another reference preparation of the same type that has been calibrated against the appropriate reference preparation listed in §630.14(a). A titration of the monovalent virus pool or the trivalent vaccine shall not constitute a valid test unless the titration of the reference virus when tested in parallel is within ± 0.5 $\log_{10}$ of its established titer. The titration of the parallel reference is intended to validate the test system and shall not be used to adjust the titer of the pool or lot under test.

(b) *Dose.* The human dose of trivalent vaccines shall be constituted to have infectivity titers in the final container material of $10^{6.0}$ to $10^{7.0}$ for type 1, $10^{5.1}$ to $10^{6.1}$ for type 2, and $10^{5.8}$ to $10^{6.8}$ for type 3, when assayed in HEp–2 cells, or the equivalent when titrated by a different method.

§ 630.16 Test for neurovirulence.

(a) Except as provided in §630.17, the following test relating to safety prescribed in paragraph (b) of this section shall be performed on each monovalent virus pool after the filtration process.

(b) *Neurovirulence in monkeys.* Except as provided in paragraph (b)(5) of this section, each monovalent virus pool shall be tested concurrently with the corresponding type Reference Attenuated Poliovirus for neurovirulence by the intraspinal route of injection in Macaca monkeys. Whenever possible the monkeys should be of comparable age and weight and from the same quarantine group. The monkeys shall be distributed randomly between the two test groups. If the number of monkeys included in both groups precludes completion during a single workday, approximately equal numbers of monkeys shall be inoculated with the monovalent virus pool and the reference preparation during each of the testing days. A preinjection serum sample obtained from each monkey shall be shown to contain no neutralizing antibody in a dilution of 1:4 when tested against no more than 1,000 $TCID_{50}$ (mean tissue culture infectious doses) of each of the three types of poliovirus. The neurovirulence test is not valid unless the inoculation sample is shown to contain the equivalent of $10^{6.5}$ to $10^{7.5}$ $TCID_{50}$ per milliliter when a representative sample of the monovalent virus pool is titrated in HEp-2 cells in comparison with the Reference Poliovirus, Live, Attenuated of the appropriate type. All monkeys shall be observed for 17 to 21 days and any evidence of physical abnormalities indicative of poliomyelitis or other viral infections shall be recorded.

(1) *Intraspinal inoculation.* For tests with type 1 and type 2 monovalent virus pools and the Reference Attenuated Poliovirus of the corresponding types, each of a group of at least 12 monkeys after being suitably anesthetized shall be injected intraspinally into the enlargement of the lumbar cord with 0.1 milliliter of the inoculation sample. For tests with type 3 poliovirus materials, groups of at least 20 monkeys shall be injected as above after being suitably anesthetized. A test of a virus pool shall include at least one group of monkeys, and no more than three groups shall be inoculated, with the results from testing one, two, or three groups of monkeys being evaluated as prescribed in §630.16(b)(2). In addition, if on examination there is no evidence of correct inoculation, additional animals may be inoculated in order to reestablish the minimum number of 11 positive monkeys for tests of types 1 and 2 virus

pools and the minimum number of 18 positive monkeys for tests of Type 3 virus pools. A positive monkey is an animal which either survives for 11 or more days or succumbs or is sacrificed due to a severe poliovirus infection at any time before the 11th day of the observation period and in which neural lesions specific for poliovirus are seen in the central nervous system. If at least 60 percent of the animals of a group survive 48 hours after inoculation, those animals that did not survive may be replaced by additional animals. If less than 60 percent of the animals in a group survive 48 hours after inoculation, the test shall be considered invalid and shall be repeated.

(2) *Determination of neurovirulence.* At the conclusion of the observation period, the animals are sacrificed and a comparative evaluation shall be made of the evidence of neurovirulence of the monovalent virus pool under test and the Reference Attenuated Poliovirus of the corresponding type with respect to the histopathology of lesions caused by poliovirus. Animals dying or sacrificed when severely paralyzed or moribund during the test period, should be included in the evaluation, except that these examinations of these monkeys shall be made immediately after death. Histopathological examinations by a qualified pathologist shall be made of at least the lumbar and cervical enlargements, the medulla, the mesencephalon, the thalamus, and motor cortex of each monkey in the groups injected with the monovalent virus pool or with the reference under test. The magnitude of the neuropathology exhibited in the lumbar and cervical areas, the medulla, and mesencephalon of all positive monkeys inoculated with the monovalent virus pool shall be quantified and compared to the magnitude of the neuropathology determined based on the same type of evaluation of monkeys in the current test and all previous tests of the Reference Attenuated Poliovirus of the corresponding type. The monovalent virus pool may be used for poliovirus vaccine if a comparative analysis of the test results demonstrates that the numerical value assigned for neurovirulence of the monovalent virus pool is equal to or less than that of the Reference Attenuated Poliovirus of the corresponding type. If the numerical value assigned for neurovirulence of the monovalent virus pool is greater than that of the Reference Atteneuated Poliovirus, the monovalent virus pool is acceptable if the difference is not greater than that calculated by a mathematical method that is expected to reject vaccines with neurovirulence identical to the reference at a frequency of not less than 1 in 100 when 1 group of monkeys is inoculated. If 2 groups are injected with the same monovalent virus pool under test, the frequency of rejection shall be not less than 5 in 100 and for 3 groups, not less than 10 in 100. If the difference in numerical values is greater than that calculated, irrespective of which reference preparation was used in the test, the monovalent virus pool shall be considered unacceptable and shall not be used for vaccine manufacture.

(3) *Outlier scores.* In the event that one or more monkeys inoculated with virus from the monovalent virus pool have individual mean lesion scores higher than that previously or concurrently associated with the Reference Attenuated Poliovirus of the corresponding type, but the monovalent virus pool meets the criteria for acceptable neurovirulence given in §630.16(b)(2), the significance of the outlier scores shall be evaluated by a method approved by the Director, Center for Biologics Evaluation and Research before the vaccine may be released for use.

(4) *Test with Reference Attenuated Poliovirus.* Except as provided in paragraph (b)(5) of this section, the Reference Attenuated Poliovirus of the appropriate type shall be tested as prescribed in paragraph (b)(1)(i) of this section concurrently with the monovalent virus pool. More than one monovalent virus pool of the same type may be tested with the same corresponding Reference Attenuated Poliovirus. Initially, a minimum of four tests by the testing laboratory of each Reference Attenuated Poliovirus is required. These tests must be such as to provide sufficient experience to define the performance of the Reference Attenuated Poliovirus and establish the variability of the assay. Each test of

the Reference Attenuated Poliovirus shall be considered acceptable and added to the previous testing experience only if the magnitude of its poliovirus neuropathology is statistically compatible with the results of all previous tests with the same reference preparations of the same type performed by the testing laboratory.

(5) *Alternative procedures in case of monkey shortage.* In the event of a shortage of test monkeys and upon approval of the Director, Center for Biologics Evaluation and Research, a monovalent virus pool may be tested without concurrent testing of the corresponding type Reference Attenuated Poliovirus. In such a case, the magnitude of the neuropathology of the monovalent virus pool shall be compared with the magnitude of the neuropathology exhibited in all previous tests of the corresponding Reference Attenuated Poliovirus.

§ 630.17 Alternative test for neurovirulence.

(a) In lieu of the neurovirulence test in § 630.16, the following test may be performed after the filtration process, on each monovalent virus pool or on each multiple thereof (monovalent lot).

(b) *Neurovirulence in monkeys.* Each monovalent virus pool or monovalent lot shall be tested in comparison with the Reference Attenuated Poliovirus, Type 1, for neurovirulence in Macaca monkeys by both the intrathalamic and intraspinal routes of injection. A preinjection serum sample obtained from each monkey must be shown to contain no neutralizing antibody in a dilution of 1:4 when tested against no more than 1,000 $TCID_{50}$ (mean tissue culture infectious dose) of each of the three types of poliovirus. The neurovirulence tests are not valid unless the sample contains at least $10^{7.6}$ $TCID_{50}$ per milliliter when titrated in HEp-2 cells in comparison with the Reference Poliovirus, Live, Attenuated of the appropriate type. All monkeys shall be observed for 17 to 21 days and any evidence of physical abnormalities indicative of poliomyelitis or other viral infections shall be recorded.

(1) *Intrathalamic inoculation.* Each of at least 30 monkeys shall be injected intracerebrally by placing 0.5 milliliter of virus pool material into the thalamic region of each hemisphere. Comparative evaluations shall be made with the virus pool under test and the Reference Attenuated Poliovirus, Type 1. Only monkeys that show evidence of inoculation into the thalamus shall be considered as having been injected satisfactorily. With respect to inoculation, a test is deemed valid if at least 24 monkeys are considered as having been injected satisfactorily. If on examination there is evidence of failure to inoculate virus pool material into the thalamus, additional monkeys may be inoculated in order to reestablish the minimum number of monkeys for the test.

(2) *Intraspinal inoculation.* Each of a group of at least five monkeys shall be injected intraspinally with 0.2 milliliter of virus pool material containing at least $10^{7.6}$ $TCID_{50}$ per milliliter when titrated in HEp-2 cells, and each monkey in additional groups of at least five monkeys shall be injected intraspinally with 0.2 milliliter of a 1:1,000 and 1:10,000 dilution, respectively, of the same virus pool material. Comparative evaluations shall be made with the virus pool under test and the reference material. Only monkeys that show microscopic evidence of inoculation into the gray matter of the lumbar cord shall be considered as having been injected satisfactorily. With respect to inoculation, a test is deemed valid if at least four monkeys per group are considered as having been injected satisfactorily. If on examination there is evidence of failure to inoculate intraspinally, additional animals may be inoculated in order to reestablish the minimum number of animals per group.

(3) *Determination of neurovirulence.* At the conclusion of the observation period comparative histopathological examinations by a qualified pathologist shall be made of the lumbar cord, cervical cord, lower medulla, upper medulla, mesencephalon and motor cortex of each monkey in the groups injected with virus under test and those injected with the Reference Attenuated Poliovirus, Type 1, except that for animals dying during the test period, these examinations shall be made immediately after death. If at least 60

percent of the animals of a group survive 48 hours after inoculation, those animals which did not survive may be replaced by an equal number of animals tested as prescribed in paragraph (b) of this section. If less than 60 percent of the animals of a group survive 48 hours after inoculation, the test must be repeated. At the conclusion of the observation the animals shall be examined to ascertain whether the distribution and histological nature of the lesions are characteristics of poliovirus infection. A comparative evaluation shall be made of the evidence of neurovirulence of the virus under test and the Reference Attenuated Poliovirus, Type 1, with respect to:

(i) The number of animals showing lesions characteristic of poliovirus infection;

(ii) The number of animals showing lesions other than those characteristic of poliovirus infection;

(iii) The severity of the lesions;

(iv) The degree of dissemination of the lesions; and

(v) The rate of occurrence of paralysis not attributable to the mechanical injury resulting from inoculation trauma. These five factors may be weighted and interpreted as the Director, Center for Biologics Evaluation and Research, or the Director's delegatees deem appropriate. Among permissible interpretations, the factors may be considered in different ways for monkeys inoculated intraspinally and for monkeys inoculated intrathalamically. Other relevant factors in addition to those listed in paragraph (b)(3)(i) through (b)(3)(v) of this section, such as public health consequences, may be considered in evaluating neurovirulence test results. The virus pool under test is satisfactory for poliovirus vaccine only if at least 80 percent of the animals in each group survive the observation period and if a comparative analysis of the test results demonstrates that the neurovirulence of the test virus pool does not exceed that of the Reference Attenuated Poliovirus, Type 1.

(4) *Test with Reference Attenuated Poliovirus.* The Reference Attenuated Poliovirus, Type 1, shall be tested as prescribed in paragraphs (b)(1) and (b)(2) of this section at least once for every 10 production lots of vaccine, except that the interval between the test of the reference and the test of any lot of vaccine shall not be greater than 3 months. The test procedure shall be considered acceptable only if lesions of poliomyelitis are seen in monkeys inoculated with the reference material at a frequency statistically compatible with all previous tests with this preparation.

§ 630.18 Additional tests for safety.

(a) *Tests prior to filtration.* Monovalent virus pools shall contain no demonstrable viable microbial agent, except for unavoidable bacteriophage and the intended attenuated live poliovirus. The vaccine shall be tested for the absence of other infectious agents, including polioviruses of other types or strains. Testing of each monovalent pool shall include the following procedures:

(1) *Inoculation of rabbits.* A minimum of 100 milliliters of each monovalent virus pool shall be tested by inoculation into at least 10 healthy rabbits, each weighing 1,500 to 2,500 grams. Each rabbit shall be injected with a total of 1.0 milliliter intradermally in multiple sites, and subcutaneously with 9.0 milliliters, of the monovalent virus pool and the animals observed for at least 3 weeks. Each rabbit that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and the brain and organs removed and examined. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the rabbits remain healthy and survive the entire period and if all the rabbits used in the test fail to show lesions of any kind at the sites of inoculation and fail to show evidence of cercopithecid herpesvirus 1 or any other viral infection.

(2) *Inoculation of adult mice.* Each of at least 20 adult mice, each weighing 15 to 20 grams, shall be inoculated intraperitoneally with 0.5 milliliter and intracerebrally with 0.03 milliliter of each monovalent virus pool. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and

examined for evidence of viral infection by direct observation and subinoculation of appropriate tissue into at least five additional mice which shall be observed for 21 days. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the mice remain healthy and survive the entire period and if all the mice used in the test fail to show evidence of lymphocytic choriomeningitis virus or other viral infection.

(3) *Inoculation of suckling mice.* Each of at least 20 suckling mice less than 24 hours old shall be inoculated intracerebrally with 0.01 milliliter and intraperitonally with 0.1 milliliter of the monovalent virus pool. The mice shall be observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by the intracerebral and intraperitoneal routes and observed daily for 14 days. In addition, a blind passage shall be made of a single pool of the emulsified tissue (minus skin and viscera) of all mice surviving the original 14–day test. The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of the mice remain healthy and survive the entire period and if all the mice used in the test fail to show evidence of Coxsackie or other viral infection.

(4) *Inoculation of guinea pigs.* Each of at least five guinea pigs, each weighting 350 to 450 grams, shall be inoculated intracerebrally with 0.1 milliliter and intraperitoneally with 5.0 milliliters on the monovalent virus pool to be tested. The animals shall be observed for at least 42 days and rectal temperatures recorded daily for the last 3 weeks of the test. Each animal that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and its tissues shall be examined both microscopically and culturally for evidence of tubercle bacilli, and by passage of tissue suspensions into at least three other guinea pigs by the intracerebral and intraperitoneal routes of inoculation for evidence of viral infection. If clinical signs suggest infection with lymphocytic choriomeningitis virus, serological tests shall be performed on blood samples of the test guinea pigs to confirm the clinical observations. Animals that die or are sacrificed during the first 3 weeks after inoculation with the monovalent virus pools shall be examined for infection with lymphocytic choriomeningitis virus. Animals that die in the final 3 weeks shall be examined both microscopically and culturally for *Mycobacterium tuberculosis.* The monovalent virus pool may be used for poliovirus vaccine only if at least 80 percent of all animals remain healthy and survive the observation period and if all the animals used in the test fail to show evidence of infection with *Mycobacterium tuberculosis* or any viral infection.

(5) *Inoculation of monkey kidney tissue cultures.* At least 500 doses or 50 milliliters, whichever is a greater volume of virus, taken either from each undiluted monovalent virus pool or, in equal proportions from individual harvests or subpools, shall be tested for simian viruses in Macaca monkey kidney tissue cultures and, in the same volume, in Cercopithecus monkey kidney tissue cultures. A dilution of the virus pool in medium not to exceed 1:4 shall be used. The area of surface growth of the cells shall be at least 3 square centimeters per milliliter of test inoculum. The test poliovirus shall be neutralized by high-titer specific antiserum of nonprimate origin. The immunizing antigens used for the preparation of antisera shall be grown in a cell line other than the cell line used for testing the vaccine. The cultures shall be observed for at least 14 days. At the end of the observation period at least one subculture of fluid from the Cercopithecus kidney cell culture shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days. The monovalent virus pool may be used for poliovirus vaccine only if all the tissue cultures fail to show evidence of the presence of simian viruses or any other viral infection.

(6) *Inoculation of human cell cultures.* At least 500 doses or 50 milliliters, whichever represents a greater volume of virus, taken from either a single

monovalent pool or, in equal proportions from individual harvests or subpools, shall be tested for the presence of measles virus in either:

(i) Primary human amnion cells,

(ii) Primary human kidney cells, or

(iii) Any other human or nonhuman cell system of comparable suspectibility to unmodified measles virus.

The virus pool shall be diluted with medium not to exceed 1:4. The area of surface growth of cells shall be at least 3 square centimeters per milliliter of test inoculum. The test material shall be neutralized with poliovirus antiserum of other than primate origin if the tissue culture cell system used is susceptible to poliovirus. The immunizing antigens used for the preparation of antiserum shall be grown in a cell line other than the cell line used for testing the vaccine. The culture shall be observed for at least 14 days. The monovalent virus pool may be used for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of measles virus or any other viral infection.

(7) *Inoculation of a rabbit kidney tissue culture.* At least 500 milliliters of virus pool, taken from either a single monovalent pool or in equal proportions from individual harvests or subpools, shall be tested in primary rabbit kidney tissue culure preparations for evidence of cercopithecid herpesvirus 1. The virus pool shall be diluted with medium not to exceed 1:4. The area of surface growth of cells shall be at least 3 square centimeters per milliliter of test inoculum. The culture shall be observed for at least 14 days. The monovalent virus pool may be used for poliovirus vaccine only if all tissue cultures fail to show evidence of the presence of herpesvirus.

(b) *Tests for in vitro markers.* In addition to the neurovirulence test required by §§ 630.16 or 630.17, the following tests relating to safety shall be performed on each monovalent virus pools after the filtration process. Tests shall be performed on each monovalent virus pool using the marker tests described below or other methods shown to be of comparable value in indentification of the attenuated strain. The test results shall demonstrate that the monovalent virus pool under test and the seed virus have substantially the same marker characteristics.

(1) *rct/40 Marker.* Attenuated strains which grow readily at 40 °C (±0.5 °C) are classified as rct/40 positive (+) in contrast to the rct/40 negative (−) strains, which show an increased growth of at least 100,000 fold at 36 °C over that obtained at 40 °C. Comparative determinations shall be made in suitable culture vessels. ≤

(2) *d Marker.* Attenuated strains which grow readily at low concentrations of bicarbonate under agar are classified as d positive (+) in contrast to the d negative (−) strains, which exhibit delayed growth under the same conditions. The cultures shall be grown in a 36 °C incubator, in suitable culture vessels in an environment of 5 percent CO_2 in air.

(c) *Final container sterility test.* The final container sterility test need not be performed provided aseptic techniques are used in the filling process.

[56 FR 21432, May 8, 1991; 56 FR 27787, June 17, 1991]

§630.19 General requirements.

(a) *Vaccine release.* No lot of trivalent vaccine shall be released by the manufacturer unless each monovalent virus pool contained therein:

(1) Has been manufactured by the same procedures;

(2) Has met the criteria of neurovirulence for monkeys prescribed in §§ 630.16(b) or 630.17(b);

(3) Has met the criteria of in vitro markers prescribed in §630.18(b); and

(4) Has been released for further manufacturing by the Director, Center for Biologics Evaluation and Research unless, at the Director's discretion, the Director determines that lot release by the Center for Biologics Evaluation and Research is not required. The protocols for all monovalent virus pools produced sequentially from the same seed and tested, in whole or in part, in accordance with §§630.16(b) or 630.17(b) shall be submitted to the Director, Center for Biologics Evaluation and Research, whether or not release of the pool for further manufacturing is requested. For monovalent virus pools not tested under §§630.16(b) or 630.17(b),

the manufacturer shall report the reasons for partial manufacture to the Director, Center for Biologics Evaluation and Research.

(b) *Labeling.* In addition to the items required by other applicable labeling provisions of this chapter, the final container label shall bear a statement indicating that liquid vaccine may not be used for more than 7 days after opening the container. Labeling may include a statement indicating that, for frozen vaccine, a maximum of 10 freeze-thaw cycles is permissible provided the total cumulative duration of thaw does not exceed 24 hours, and provided the temperature does not exceed 8 °C during the periods of thaw.

(c) *Samples and protocols.* For each trivalent lot of vaccine and for each monovalent virus pool, the following materials shall be submitted in accordance with instructions received from the Director, Center for Biologics Evaluation and Research, 8800 Rockville Pike, Bethesda, MD 20892.

(1) A protocol that consists of a summary of the history of manufacture of each trivalent lot or monovalent virus pool, including any test results requested by the Director, Center for Biologics Evaluation and Research.

(2) Twenty milliliters of monovalent virus pool before filtration.

(3) Forty milliliters of monovalent virus pool after filtration. The titer of the sample shall be no less than the equivalent of $10^{7.5}$ $TCID_{50}$ per milliliter when titrated in HEp–2 cells; if the titer is greater than $10^{7.5}$ $TCID_{50}$ per milliliter, a correspondingly smaller volume may be submitted.

(4) A total of at least 50 single doses or the equivalent thereof of the trivalent vaccine.

(5) When deemed appropriate, the Director, Center for Biologics Evaluation and Research, may require submission of samples or sample volumes other than those specified in paragraphs (c)(2), (c)(3), and (c)(4) of this section.

(d) *Public health implications.* In interpreting any provision of the regulations governing oral poliovirus vaccine, the agency may consider any potential effect on individual or public health, including effects related to vaccine supply.

(e) *Alternative procedures.* (1) The Director, Center for Biologics Evaluation and Research, may approve an exception or alternative to any requirement in subpart B of part 630 regarding Poliovirus Vaccine Live Oral. Requests for such exceptions or alternatives should ordinarily be made in writing. However, in limited circumstances such requests may be made orally and permission may be given orally by the Director, Center for Biologics Evaluation and Research. Oral requests and approvals must be followed by written requests and written approvals.

(2) FDA will publish a list of approved alternative procedures and exceptions periodically in the FEDERAL REGISTER.

(f) *Status of vaccine in distribution.* Poliovirus Vaccine Live Oral released or in distribution prior to May 8, 1991, is deemed to meet the requirements of supart B of part 630.

Subpart C—[Reserved]

Subpart D—Measles Virus Vaccine Live

§ 630.30 Measles Virus Vaccine Live.

(a) *Proper name and definition.* The proper name of this product shall beMeasles Virus Vaccine Live, which shall consist of a preparation of live, attenuated, measles virus.

(b) *Criteria for acceptable strains of attenuated measles virus.* Strains of attenuated measles virus used in the manufacture of vaccine shall be identified by (1) historical records, including origin and manipulation during attenuation and (2) antigenic specificity as measles virus as demonstrated by tissue culture neutralization tests. Strains used for the manufacture ofMeasles Virus Vaccine Live, shall have been shown to be safe and potent in man by field studies with experimental vaccines. The vaccine shall have been demonstrated as safe and potent in at least 10,000 susceptible persons. Susceptibility shall be shown by the absence of neutralizing or other antibodies against measles virus, or by other appropriate methods. Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous

viable microbial agents except for unavoidable bacteriophage.

(c) *Neurovirulence safety test of the virus seed strain in monkeys*—(1) *The test.* A demonstration shall be made in monkeys of the lack of neurotropic properties of the seed strain of attenuated measles virus used in the manufacture of measles virus vaccine. For this purpose and to establish consistency of manufacture of the vaccine, vaccine from each of five consecutive lots shall be tested separately in the following manner:

(i) Samples of each of the five lots of vaccine shall be tested in measles susceptible monkeys. Immediately prior to initiation of a test each monkey shall have been shown to be serologically negative for neutralizing antibodies by means of a tissue culture neutralization test with undiluted serum from each monkey tested at approximately 100 $TCID_{50}$ of Edmonston strain measles virus, or negative for measles virus antibodies as demonstrated by tests of equal sensitivity.

(ii) A test sample of vaccine removed after clarification but before final dilution for standardization of virus content shall be used for the test.

(iii) Vaccine shall be injected by combined intracerebral, intraspinal, and intramuscular routes into not less than 20 Macaca or Cercopithecus monkeys or a species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable for the purpose. The animals shall be in overt good health and injected under deep barbiturate anesthesia. The intramuscular injection shall consist of 1.0 milliliter of test sample into the right leg muscles. At the same time, 200 milligrams of cortisone acetate shall be injected into the left leg muscles, and 1.0 milliliter of procaine penicillin (300,000 units) into the right arm muscles. The intracerebral injection shall consist of 0.5 milliliter of test sample into each thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 milliliter of test sample into the lumber spinal cord enlargement.

(iv) The monkeys shall be observed for 17–21 days and symptoms of paralysis as well as other neurologic disorders shall be recorded.

(v) At least 90 percent of the test animals must survive the test period without losing more than 25 percent of their weight except that, if at least 70 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48-hour test period may be replaced by an equal number of qualified test animals which are tested pursuant to paragraphs (c)(1)(i) through (iv) of this section. At least 80 percent of the injected animals surviving beyond the first 48 hours must show gross or microscopic evidence of inoculation trauma in the thalamic area and microscopic evidence of inoculation trauma in the lumbar region of the spinal cord. If less than 70 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals meet the inoculation criteria prescribed in this paragraph, the test must be repeated.

(vi) At the end of the observation period, each surviving monkey shall (*a*) be bled and the serum tested for evidence of serum antibody conversion to measles virus and (*b*) be autopsied and samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identification if needed pursuant to paragraph (c)(1)(vii) of this section. Histological sections shall be prepared from both spinal cord enlargements and appropriate sections of the brain and examined.

(vii) Doubtful histopathological findings necessitate (*a*) examination of a sample of sections from several regions of the brain in question, and (*b*) attempts at virus recovery from the nervous systems tissues previously removed from the animal.

(viii) The lot is satisfactory if the histological and other studies demonstrate no evidence of changes in the central nervous system attributable to unusual neurotropism of the seed virus or of the presence of extraneous neurotropic agents.

(2) *Wild virus controls.* As a check against the inadvertent introduction of wild measles virus, at least four uninoculated measles susceptible control monkeys shall be maintained as either cage mates to, or within the same immediate area of, the 20 inoculated test animals for each lot of vaccine for

the entire period of observation (17–21 days) and an additional 10 days. Serum samples from these control contact monkeys drawn at the time of seed virus inoculation of the test animals, and again after completion of the test, shall be shown to be free of measles neutralizing antibodies.

(3) *Test results.* (i) For each lot of vaccine under test, at least 80 percent of the monkeys must show measles antibody serological conversion (1:4 or greater) when the serum as obtained from the monkey is tested and the control contact monkeys must demonstrate no immunological response indicative of measles virus infection.

(ii) The measles virus seed has acceptable neurovirulence properties for use in vaccine manufacture only if for each of the five lots (*a*) 90 percent of the monkeys survive the observation period, (*b*) the histological and other studies produce no evidence of changes in the central nervous system attributable to unusual neurotropism of the seed virus, and (*c*) there is no evidence of the presence of extraneous neurotropic agents.

(4) *Need for additional neurovirulence safety testing.* A neurovirulence safety test as prescribed in this paragraph shall be performed on vaccine from five consecutive lots whenever a new production seed lot is introduced or whenever the source of cell culture substrate must be reestablished and recertified as prescribed in §630.32(a) and (b) of this part.

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 49 FR 23834, June 8, 1984; 50 FR 4138, Jan. 29, 1985; 55 FR 11013, Mar. 26, 1990; 55 FR 47875, Nov. 16, 1990]

§630.31 Clinical trials to qualify for license.

To qualify for license, the antigenicity of the vaccine shall have been determined by clinical trials of adequate statistical design, by a suitable route of administration of the product. Such clinical trials shall be conducted with five lots of measles virus vaccine which have been manufactured by the same methods. There shall be a demonstration under circumstances in which adequate clinical and epidemiological surveillance of illness has been maintained to show that the measles virus vaccine, when administered as recommended by the manufacturer, is free of harmful effect upon administration to approximately 1,000 susceptible individuals, in that there were no detectable neutralizing antibodies before vaccination and there was serological conversion after vaccination. The five lots of vaccine shall be distributed as evenly as possible among the 1,000 individuals tested. Demonstration shall be made of immunogenic effect by the production of specific measles neutralizing antibodies (i.e., sero-conversion from less than 1:4 to 1:8 or greater) in at least 90 percent of each of five groups of measles susceptible individuals, each having received a virus vaccine dose which is not greater than that which was demonstrated to be safe in field studies (§630.30(b)) when used under comparable conditions. Such clinical trials shall be conducted in compliance with part 56 of this chapter unless exempted under §56.104 or granted a waiver under §56.105, and with the requirements for informed consent set forth in part 50 of this chapter.

[55 FR 47875, Nov. 16, 1990]

§630.32 Manufacture of live, attenuated Measles Virus Vaccine.

(a) *Virus cultures.* Virus shall be propagated in chick embryo tissue cultures.

(b) *Virus propagated in chick embryo tissue cultures.* Embryonated chicken eggs used as the source of chick embryo tissue for the propagation of measles virus shall be derived from flocks certified to be free of Salmonella pullorum, avian tuberculosis, fowl pox, Rous sarcoma, avian leucosis, reticuloendotheliosis virus, and other adventitious agents pathogenic for chickens. If eggs are procured from flocks that are not so certified, tests shall be performed to demonstrate freedom of the vaccine from such agents. (See §630.35(a)(8) for test for avian leucosis.)

(c) [Reserved]

(d) *Passage of virus strain in vaccine manufacture.* Virus in the final vaccine shall represent no more than ten tissue culture passages beyond the passage used to perform the clinical trials (§630.30(b)) which qualified the manufacturer's vaccine strain for license.

(e) *Tissue culture preparation.* Only primary cell tissue cultures shall be used in the manufacture of Measles Virus Vaccine. Continuous cell lines shall not be introduced or propagated in Measles Virus Vaccine manufacturing areas.

(f) *Control vessels.* (1) From the tissue used for the preparation of tissue cultures for growing attenuated measles virus, an amount of processed cell suspension equivalent to that used to prepare 500 ml. of tissue culture shall be used to prepare uninfected tissue control materials. This material shall be distributed in control vessels and observed microscopically for a period of no less than 14 days beyond the time of inoculation of the production vessels with measles virus; but if the production vessels are held for use in vaccine manufacture for more than 14 days, the control vessels shall be held and observed for the additional period. At the end of the observation period or at the time of virus harvest, whichever is later, fluids from the control cultures shall be tested for the presence of adventitious agents as follows:

Samples of fluid from each control vessel shall be collected at the same time as fluid is harvested from the corresponding production vessels. If multiple virus harvests are made from the same cell suspension, the control samples for each harvest shall be frozen and stored at −60° C. until the last viral harvest for that cell suspension is completed. The fluid from all the control samples from that suspension shall be pooled in proportionate amounts and at least five ml. inoculated into human and simian cell tissue culture systems and in the tissue culture system used for virus production. The cultures shall be observed for the presence of changes attributable to growth of adventitious viral agents including hemadsorption viral agents.

(2) The cell sheets of one quarter to one third of the control vessels shall be examined at the end of the observation period (14 days or longer) for the presence of hemadsorption viruses by the addition of guinea pig red blood cells. If the chick embryo cultures were not derived from a certified source (paragraph (b) of this section), the remaining tissue culture controls may be used to test for avian leucosis virus using either Rubin's procedure for detecting Resistance Inducing Factor (RIF) or a method of equivalent effectiveness.

(3) The test is satisfactory only if there is no evidence of adventitious viral agents and if at least 80 percent of the control vessels are available for observation at the end of the observation period (14 days or longer).

(g) *Test samples.* Samples of virus harvests or pools for testing by inoculation into animals, into tissue culture systems, into embryonated hens' eggs, and into bacteriological media, shall be withdrawn immediately after harvesting or pooling but prior to freezing except that samples of test materials frozen immediately after harvesting or pooling and maintained at −60° C. or below, may be tested upon thawing, provided no more than two freeze-thaw cycles are employed. The required tests shall be initiated without delay after thawing.

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 47 FR 24699, June 8, 1982]

§ 630.33 Reference virus.

A U.S. Reference Measles Virus, Live, Attenuated, shall be obtained from the Center for Biologics Evaluation and Research as a control for correlation of virus titers.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 630.34 Potency test.

The concentration of live measles virus shall constitute the measure of potency. The titration shall be performed in a suitable cell culture system, free of wild viruses, using either the U.S. Reference Measles Virus, Live, Attenuated or a calibrated equivalent strain as a titration control. The concentration of live measles virus contained in the vaccine of each lot under test shall be no less than the equivalent of 1,000 $TCID_{50}$ of the U.S. reference per human dose.

§ 630.35 Test for safety.

(a) *Tests prior to clarification of vaccine manufactured in chick embryo tissue cultures.* Prior to clarification, the following tests shall be performed on each virus pool of chick embryo tissue culture:

(1) *Inoculation of adult mice.* Each of at least 20 adult mice each weighing 15–20 grams shall be inoculated intraperitoneally with 0.5 ml. and intracerebrally with 0.03 ml. amounts of each virus pool to be tested. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection by direct observation and subinoculation of appropriate tissue into at least five additional mice which shall be observed for 21 days. The virus pool may be used only if at least 80 percent of the original group of mice remain healthy and survive the observation period and if none of the mice show evidence of a transmissible agent or other viral infection, other than measles virus, attributable to the vaccine.

(2) *Inoculation of suckling mice.* Each of at least 20 suckling mice less than 24 hours old shall be inoculated intracerebrally with 0.01 ml. and intraperitoneally with 0.1 ml. of the virus pool to be tested. The mice shall be observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by intracerebral and intraperitoneal routes and observed daily for 14 days. In addition, a blind passage shall be made of a single pool of the emulsified tissue (minus skin and viscera) of all mice surviving the original 14-day test. The virus pool is satisfactory for Measles Virus Vaccine only if at least 80 percent of the original inoculated mice remain healthy and survive the entire observation period, and if none of the mice used in the test show evidence of a transmissible agent or viral infection, other than measles virus, attributable to the vaccine.

(3) *Inoculation of monkey tissue cell cultures.* A volume of virus suspension of each undiluted virus pool, equivalent to at least 500 human doses or 50 milliliters, whichever represents a greater volume, shall be tested for adventitious agents in *Cercopithecus* monkey kidney tissue culture preparations or *Erythrocebus patas* monkey kidney tissue culture preparations, after neutralization of the measles virus by a high titer antiserum of nonhuman, nonsimian and nonchicken origin. The immunizing antigen used for the preparation of the measles antiserum shall be grown in tissue culture cells that shall be free of extraneous viruses which might elicit antibodies that could inhibit growth of extraneous viruses present in the measles virus pool. The tissue culture of the virus pool shall be observed for no less than 14 days. The virus pool is satisfactory for measles virus vaccine only if all the tissue culture tests fail to show evidence of any extraneous transmissible agent other than measles virus attributable to the vaccine.

(4) *Inoculation of other cell cultures.* The measles virus pool shall be tested in the same manner as prescribed in paragraph (a)(3) of this section in rhesus or cynomolgus monkey kidney, chick embryo, and human tissue cell cultures.

(5) *Inoculation of embryonated chicken eggs.* A volume of virus suspension of each undiluted virus pool, equivalent to at least 100 doses or 10 milliliters, whichever represents a greater volume, after neutralization of the measles virus by a high titer antiserum of nonhuman, nonsimian, nonavian origin shall be tested as follows:

(i) Embryonated eggs, 10 to 11 days old, shall be inoculated by the allantoic route using 0.5 milliliter per egg. Follow incubation at 35° C for 72 hours, the allantoic fluids shall be harvested, pooled, and subpassed by the same route into fresh, embryonated eggs, 10 to 11 days old, using 0.5 milliliter per egg and incubated at 35° C for 72 hours. Both the initial pool and the subpassage harvest shall be tested for the presence of hemagglutinin. The virus pool is satisfactory if the embryos appear normal and there is no evidence of hemagglutinating agents.

(ii) Embryonated eggs, 6 to 7 days old, shall be inoculated by the yolk sac route using 0.5 milliliter per egg. Following incubation at 35° C for at least 9 days, the yolk sacs shall be harvested and pooled. A 10-percent suspension of

yolk sacs shall be subpassed by the same route into fresh embryonated eggs, 6 to 7 days old, using 0.5 milliliter of inoculum per egg and incubated at 35° C for at least 9 days. The virus pool is satisfactory if the embryos in both the initial test and the subpassage appear normal.

(6) [Reserved]

(7) *Bacteriological tests.* Each virus pool shall be tested for sterility in accordance with §610.12 of this chapter. In addition each virus pool shall be tested for the presence of M. tuberculosis, both avian and human, by appropriate culture methods.

(8) *Test for avian leucosis.* If the cultures were not derived from a certified source (§630.32(b)), and the control fluids were not tested for avian leucosis (§630.32(f)), at least 500 doses or 50 ml., whichever represents a greater volume of each undiluted vaccine pool, shall be tested and found negative for avian leucosis, using either Rubin's procedure for detecting Resistance Inducing Factor (RIF) or another method of equivalent effectiveness.

(b) [Reserved]

(c) *Clarification.* After harvesting and removal of samples for testing as prescribed above in this section, the virus fluids shall be clarified by centrifugation, by passage through filters of sufficiently small porosity, or by any other method that will assure removal of all intact tissue cells which may have been collected in the harvesting process.

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 41 FR 43400, Oct. 1, 1976; 47 FR 24699, June 8, 1982]

§ 630.36 General requirements.

(a) *Final container tests.* In addition to the tests required pursuant to §610.14 of this chapter, an immunological and virological identity test shall be performed on the final container if it was not performed on each pool or the bulk vaccine prior to filling.

(b)—(c) [Reserved]

(d) *Dose.* These standards are based on an individual human immunizing dose of no less than 1,000 $TCID_{50}$ of Measles Virus Vaccine Live, expressed in terms of the assigned titer of the U.S. reference measles virus.

(e) *Labeling.* In addition to the items required by other applicable labeling provisions of this subchapter, single-dose container labeling for vaccine which is not protected against photochemical deterioration shall include a statement cautioning against exposure to sunlight.

(f) [Reserved]

(g) *Photochemical deterioration; protection.* Vaccine in multiple dose final containers shall be protected against photochemical deterioration. Such containers may be colored, or outside coloring or protective covering may be used for this purpose, provided (1) the method used is shown to provide the required protection, and (2) visible examination of the contents is not precluded. Vaccine in single dose containers may be protected in the same manner provided the same conditions are met.

(h) *Sample and protocols.* The following materials shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892:

(1) For each lot of vaccine:

(i) A protocol which consists of a summary of the history of the manufacture of the lot, including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.

(ii) A total of no less than two 25-milliliter volumes in a frozen state (−60° C) of preclarification bulk vaccine containing no preservative or adjuvant.

(iii) A total of no less than 30 containers of the vaccine from each filling of each bulk lot of single-dose containers. A total of no less than six 50-dose containers or ten 10-dose containers of the vaccine from each filling of each bulk lot of multiple-dose containers.

(2) In addition to the requirements of paragraph (h)(1) of this section, whenever a new production seed lot is introduced, or whenever the source of cell culture substrate must be reestablished and recertified, samples consisting of no less than 100 milliliters in 10 milliliter volumes, in a frozen state (−60° C), of postclarification bulk vaccine

containing stabilizer but no preservative or adjuvant, taken from each of 5 consecutive lots of the bulk vaccine.

[38 FR 32068, Nov. 20, 1973, as amended at 41 FR 10429, Mar. 11, 1976; 49 FR 23834, June 8, 1984; 50 FR 4138, Jan. 29, 1985; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

Subpart E—[Reserved]

Subpart F—Mumps Virus Vaccine Live

§ 630.50 Mumps Virus Vaccine Live.

(a) *Proper name and definition.* The proper name of this product shall be Mumps Virus Vaccine Live, which shall consist of a preparation of live, attenuated mumps virus.

(b) *Criteria for acceptable strains of attenuated mumps virus.* Strains of attenuated mumps virus used in the manufacture of vaccine shall be identified by (1) historical records including origin and manipulation during attenuation, (2) antigenic specificity as mumps virus as demonstrated by tissue culture neutralization tests. Strains used for the manufacture of Mumps Virus Vaccine Live shall have been shown to be safe and potent in at least 5,000 susceptible individuals by field studies with experimental vaccines. Susceptibility shall be shown by the absence of neutralizing or other antibodies against mumps virus, or by other appropriate methods. Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous viable microbial agents except for unavoidable bacteriophage.

(c) *Neurovirulence safety test of the virus seed strain in monkeys*—(1) *The test.* A demonstration shall be made in monkeys of the lack of neurotropic properties of the seed strain of attenuated mumps virus used in the manufacture of mumps vaccine. For this purpose and to establish consistency of manufacture of the vaccine, vaccine from each of five consecutive lots shall be tested separately in monkeys shown to be serologically negative for mumps virus antibodies in the following manner:

(i) A test sample of vaccine removed after clarification but before final dilution for standardization of virus content shall be used for the test.

(ii) Vaccine shall be injected by combined intracerebral, intraspinal, and intramuscular routes into not less than 20 Macaca or Cercopithecus monkeys or a species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable for the purpose. The animals shall be in overt good health and injected under deep barbiturate anesthesia. The intramuscular injection shall consist of 1.0 milliliter of test sample into the right leg muscles. At the same time, 200 milligrams of cortisone acetate shall be injected into the left leg muscles, and 1.0 milliliter of procaine penicillin (300,000 units) into the right arm muscles. The intracerebral injection shall consist of 0.5 milliliter of test sample into each thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 milliliter of test sample into the lumbar spinal cord enlargement.

(iii) The monkeys shall be observed for 17–21 days and symptoms of paralysis as well as other neurologic disorders shall be recorded.

(iv) At least 90 percent of the test animals must survive the test period without losing more than 25 percent of their weight except that, if at least 70 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48-hour test period may be replaced by an equal number of qualified test animals which are tested pursuant to paragraphs (c)(1)(i) through (iii) of this section. At least 80 percent of the injected animals surviving beyond the first 48 hours must show gross or microscopic evidence of inoculation trauma in the thalamic area and microscopic evidence of inoculation trauma in the lumbar region of the spinal cord. If less than 70 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals meet the inoculation criteria prescribed in this paragraph, the test must be repeated.

(v) At the end of the observation period, each surviving animal shall be autopsied and samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identification if

needed pursuant to paragraph (c)(1) (vi) of this section. Histological sections shall be prepared from both spinal cord enlargements and appropriate sections of the brain and examined.

(vi) Doubtful histopathological findings necessitate (*a*) examination of a sample of sections from several regions of the brain in question, and (*b*) attempts at virus recovery from the nervous system tissues previously removed from the animals.

(vii) The lot is satisfactory if the histological and other studies demonstrate no evidence of changes in the central nervous system attributable to unusual neurotropism of the seed virus or of the presence of extraneous neurotropic agents.

(2) *Test results.* The mumps virus seed has acceptable neurovirulence properties for use in vaccine manufacture only if for each of the five lots (i) 90 percent of the monkeys survive the observation period, (ii) the histological and other studies produce no evidence of changes in the central nervous system attributable to unusual neurotropism or replication of the seed virus and (iii) there is no evidence of the presence of extraneous neurotropic agents.

(3) *Need for additional neurovirulence safety testing.* A neurovirulence safety test as prescribed in this paragraph shall be performed on vaccine from five consecutive lots whenever a new production seed lot is introduced or whenever the source of cell culture substrate must be reestablished and recertified as prescribed in §630.52(a).

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 50 FR 4138, Jan. 29, 1985; 55 FR 11013, Mar. 26, 1990; 55 FR 47875, Nov. 16, 1990]

§630.51 Clinical trials to qualify for license.

To qualify for license, the antigenicity of Mumps Virus Vaccine Live shall be determined by clinical trials, conducted in compliance with part 56 of this chapter unless exempted under §56.104 or granted a waiver under §56.105, and with part 50 of this chapter, that follow the procedures prescribed in §630.31, except that the immunogenic effect shall be demonstrated by establishing that a protective antibody response has occurred in at least 90 percent of each of the five groups of mumps-susceptible individuals, each having received the parenteral administration of a virus vaccine dose not greater than that demonstrated to be safe in field studies (§630.50(b)) when used under comparable conditions.

[46 FR 8956, Jan. 27, 1981, as amended at 50 FR 4138, Jan. 29, 1985]

§630.52 Manufacture of Mumps Virus Vaccine Live

(a) *Virus cultures.* Mumps virus shall be propagated in chick embryo cell cultures. The embryonated chicken eggs used as the source of chick embryo tissue for the propagation of mumps virus shall be derived from flocks certified or tested as prescribed in §630.32(b).

(b) *Passage of virus strain in vaccine manufacture.* Virus in the final vaccine shall represent no more than five cell culture passages beyond the passage used to perform the clinical trials (§630.50(b)) which qualified the manufacturer's vaccine strain for license.

(c) *Cell culture preparation.* Only primary cell cultures shall be used in the manufacture of mumps virus vaccine. Continuous cell lines shall not be introduced or propagated in mumps virus vaccine manufacturing areas.

(d) *Control vessels.* From the tissue used for the preparation of cell cultures for growing attenuated mumps virus, an amount of processed cell suspension equivalent to that used to prepare 500 ml. of cell culture shall be used to prepare uninfected tissue control materials which shall be prepared and tested by following the procedures prescribed in §630.32(f).

(e) *Test samples.* Test samples of mumps virus harvests or pools shall be withdrawn and maintained by following the procedures prescribed in §630.32(g).

[38 FR 32068, Nov. 20, 1973, as amended at 50 FR 4138, Jan. 29, 1985]

§630.53 Reference virus.

An NIH Reference Mumps Virus, Live, shall be obtained from the Center for Biologics Evaluation and Research

as a control for correlation of virus titers.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 630.54 Potency test.

The concentration of live mumps virus shall constitute the measure of potency. The titration shall be performed in a suitable cell culture system, free of wild viruses, using either the Reference Mumps Virus, Live, or a calibrated equivalent strain as a titration control. The concentration of live mumps virus contained in the vaccine of each lot under test shall be no less than the equivalent of 5,000 $TCID_{50}$ of the reference virus per human dose.

§ 630.55 Test for safety.

(a) *Tests prior to clarification.* Prior to clarification, the following tests shall be performed on each mumps virus pool prepared in chick embryo cell culture:

(1) *Inoculation of adult mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(1), and the virus pool is satisfactory only if equivalent test results are obtained.

(2) *Inoculation of suckling mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(2), and the virus pool is satisfactory only if equivalent test results are obtained.

(3) *Inoculation of monkey cell cultures.* A mumps virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), and the virus pool is satisfactory only if equivalent test results are obtained.

(4) *Inoculation of other cell cultures.* The mumps virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), in rhesus or cynomolgus monkey kidney, in whole chick embryo, and in human cell cultures. In addition, each virus pool shall be tested in chick embryo kidney in the same manner except that the volume tested in each cell culture shall be equivalent to 250 human doses or 25 milliliters, whichever represents a greater volume. The mumps virus pool is satisfactory only if results equivalent to those in § 630.35(a)(3) are obtained.

(5) *Inoculation of embryonated chicken eggs.* A neutralized suspension of each undiluted mumps virus pool shall be tested in the volume and following the procedures prescribed in § 630.35(a)(5), and the virus pool is satisfactory only if there is no evidence of adventitious agents.

(6) *Bacteriological tests.* In addition to the tests for sterility required pursuant to § 610.12 of this chapter, bacteriological tests shall be performed on each mumps virus pool for the presence of M. tuberculosis, both avian and human, by appropriate culture methods. The virus pool is satisfactory only if found negative for M. tuberculosis, both avian and human.

(7) *Test for avian leucosis.* If the cultures were not derived from a certified source and control fluids were not tested for avian leucosis, the vaccine shall be tested in the volume and following the procedures prescribed in § 630.35(a)(8). The cultures are satisfactory for vaccine manufacture if found negative for avian leucosis.

(b) *Clarification.* The mumps virus fluids shall be clarified by following the procedures prescribed in § 630.35(c).

[38 FR 32068, Nov. 20, 1973, as amended at 55 FR 47876, Nov. 16, 1990]

§ 630.56 General requirements.

(a) *Final container tests.* In addition to the tests required pursuant to § 610.14 of this chapter, an immunological and virological identity test shall be performed on the final container if it was not performed on each pool or the bulk vaccine prior to filling.

(b) *Dose.* These standards are based on an individual human immunizing dose of no less than 5,000 $TCID_{50}$ of Mumps Virus Vaccine Live, expressed in terms of the assigned titer of the Reference Mumps Virus, Live.

(c) *Labeling.* In addition to the items required by other applicable labeling provisions of this part, single dose container labeling for vaccine which is not protected against photochemical deterioration shall include a statement cautioning against exposure to sunlight.

(d) [Reserved]

(e) *Photochemical deterioration; protection.* Mumps Virus Vaccine Live, in multiple dose containers, shall be protected against photochemical deterioration in accordance with the procedures prescribed in § 630.36(g).

(f) *Samples and protocols.* For each lot of vaccine, the following materials shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892:

(1) A protocol which consists of a summary of the history of manufacture of each lot including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.

(2) A total of no less than two 25-milliliter volumes, in a frozen state (−60° C), of preclarification bulk vaccine containing no preservative, stabilizer, or adjuvant.

(3) A total of no less than 30 containers of the vaccine from each filling of each bulk lot of single-dose containers. A total of no less than six 50-dose containers or ten 10-dose containers of the vaccine from each filling of each bulk lot of multiple-dose containers.

[38 FR 32068, Nov. 20, 1973, as amended at 39 FR 9661, Mar. 13, 1974; 41 FR 10429, Mar. 11, 1976; 49 FR 23834, June 8, 1984; 50 FR 4138, Jan. 29, 1985; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

Subpart G—Rubella Virus Vaccine Live

§ 630.60 Rubella Virus Vaccine Live.

(a) *Proper name and definition.* The proper name of this product shall be Rubella Virus Vaccine Live, which shall consist of a preparation of live, attenuated rubella virus.

(b) *Criteria for acceptable strains of attenuated rubella virus.* Strains of attenuated rubella virus used in the manufacture of vaccine shall be identified by (1) historical records including origin and manipulation during attenuation and (2) antigenic specificity as rubella virus as demonstrated by tissue culture neutralization tests.

(c) *Extraneous agents.* Seed virus used for vaccine manufacture shall be free of all demonstrable extraneous viable microbial agents except for unavoidable bacteriophage.

(d) *Field studies with experimental vaccines.* (1) Strains used for the manufacture of Rubella Virus Vaccine Live, shall have been shown in field studies with experimental vaccines to be safe and potent in the group of individuals inoculated, which must include at least 10,000 susceptible individuals. Susceptibility shall be shown by the absence of neutralizing or hemagglutination-inhibiting antibodies against rubella virus or by other appropriate methods.

(2) The virus strain used in the field studies shall be propagated in the same cell culture system that will be used in the manufacture of the product.

(3) The field studies shall be so conducted that at least 5,000 of the susceptible individuals must reside when inoculated in areas where health related statistics are regularly compiled in accordance with procedures such as those used by the National Center for Health Statistics. Data in such form as will identify each inoculated person shall be furnished to the Director, Center for Biologics Evaluation and Research.

(4) Inoculated persons shall be shown not to be contagious for contacts through surveillance of rubella susceptible contacts of the inoculated persons.

(e) *Neurovirulence safety test of the virus seed strain in monkeys*—(1) *The test.* A demonstration shall be made in monkeys of the lack of neurotropic properties of the seed strain of attenuated rubella virus used in the manufacture of rubella vaccine. For this purpose and to establish consistency of manufacture of the vaccine, vaccine from each of five consecutive lots shall be tested separately in monkeys shown to be serologically negative for rubella virus antibodies in the following manner:

(i) A test sample of vaccine removed after clarification but before final dilution for standardization of virus content shall be used for the test.

(ii) Vaccine shall be injected by combined intracerebral, intraspinal, and intramuscular routes into not less than 20 Macaca or Cercopithecus monkeys or a species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable for the purpose. The animals shall be in overt

good health and injected under deep barbiturate anesthesia. The intramuscular injection shall consist of 1.0 milliliter of test sample into the right leg muscles. At the same time, 200 milligrams of cortisone acetate shall be injected into the left leg muscles, and 1.0 milliliter of procaine penicillin (300,000 units) into the right arm muscles. The intracerebral injection shall consist of 0.5 milliliter of test sample into each thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 milliliter of test sample into the lumbar spinal cord enlargement.

(iii) The monkeys shall be observed for 17–21 days and symptoms of paralysis as well as other neurologic disorders shall be recorded.

(iv) At least 90 percent of the test animals must survive the test period without losing more than 25 percent of their weight except that, if at least 70 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48-hour test period may be replaced by an equal number of qualified test animals which are tested pursuant to paragraphs (e)(1)(i) through (iii) of this section. At least 80 percent of the injected animals surviving beyond the first 48 hours must show gross or microscopic evidence of inoculation trauma in the thalamic area and microscopic evidence of inoculation trauma in the lumbar region of the spinal cord. If less than 70 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals meet the inoculation criteria prescribed in this paragraph, the test must be repeated.

(v) At the end of the observation period, each surviving animal shall be autopsied and samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identification if needed pursuant to paragraph (e)(1) (vi) of this section. Histological sections shall be prepared from both spinal cord enlargements and appropriate sections of the brain and examined.

(vi) Doubtful histopathological findings necessitate (*a*) examination of a sample of sections from several regions of the brain in question, and (*b*) attempts at virus recovery from the nervous system tissues previously removed from the animal.

(vii) The lot is satisfactory if the histological and other studies demonstrate no evidence of changes in the central nervous system attributable to the presence of unusual neurotropism of the seed virus or of the presence of extraneous neurotropic agents.

(2) *Test results.* The rubella virus seed has acceptable neurovirulence properties for use in vaccine manufacture only if for each of the five lots: (i) 90 percent of the monkeys survive the observation period, (ii) the histological and other studies produce no evidence of changes in the central nervous system attributable to the presence of unusual neurotropism or replication of the seed virus and (iii) there is no evidence of the presence of extraneous neurotropic agents.

(3) *Need for additional neurovirulence safety testing.* A neurovirulence safety test as prescribed in this paragraph shall be performed on vaccine from five consecutive lots whenever a new production seed lot is introduced or whenever the source of cell culture substrate must be reestablished and recertified as prescribed in §630.62(a), (b) and (d) of this part.

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 49 FR 23834, June 8, 1984; 50 FR 4138, Jan. 29, 1985; 55 FR 11013, Mar. 26, 1990; 55 FR 47876, Nov. 16, 1990]

§ 630.61 Clinical trials to qualify for license.

To qualify for license, the antigenicity of Rubella Virus Vaccine Live, shall be determined by clinical trials, conducted in compliance with part 56 of this chapter unless exempted under §56.104 or granted a waiver under §56.105, and with part 50 of this chapter, that follow the procedures prescribed in §630.31, except that the immunogenic effect shall be demonstrated by establishing that a protective antibody response has occurred in at least 90 percent of each of the five groups of rubella-susceptible individuals, each having received the parenteral administration of a virus vaccine dose not greater than that demonstrated to be safe in field studies

when used under comparable conditions.

[46 FR 8956, Jan. 27, 1981, as amended at 50 FR 4138, Jan. 29, 1985]

§630.62 Production.

(a) *Virus cultures.* Rubella virus shall be propagated in duck embryo cell cultures, rabbit renal cultures, or in a cell line found by the Director, Center for Biologics Evaluation and Research, to meet the requirements of §610.18(c) of this chapter.

(b) *Virus propagated in duck embryo tissue cell cultures.* Embryonated duck eggs used as a source of duck embryo tissue for the propagation of rubella virus shall be derived from flocks certified to be free of avian tuberculosis, the avian leucosis-sarcoma group of viruses, reticuloendotheliosis virus, and other agents pathogenic for ducks. Only ducks so certified and in overt good health and which are maintained in quarantine shall be used as a source of duck embryo tissue used in the propagation of rubella virus. Ducks in the quarantined flock that die shall be necropsied and examined for evidence of significant pathologic lesions. If any such signs or pathologic lesions are observed, eggs from that flock shall not be used for the manufacture of Rubella Virus Vaccine Live. Control vessels shall be prepared, observed, and tested as prescribed in §630.32(f).

(c) [Reserved]

(d) *Virus propagated in rabbit renal tissue cell cultures.* Only rabbits in overt good health which have been maintained in quarantine individually caged in vermin-proof quarters for a minimum of 6 months, having had no exposure to other rabbits or animals throughout the quarantine period, or rabbits born to rabbits while so quarantined, provided the progency have been kept in the same type of quarantine continuously from birth shall be used as a source of kidney tissue. Animals shall be free of antibodies for agents potentially pathogenic for man unless it has been demonstrated in the license application that the tests required by §630.65(c) to be performed on each lot of vaccine are capable of detecting contamination of agents capable of producing such antibodies.

(1) *Rabbits used for experimental purposes.* Rabbits that have been used previously for experimental or testing purposes with microbiological agents shall not be used as a source of kidney tissue in the production of vaccine.

(2) *Quarantine and necropsy.* Each rabbit shall be examined periodically during the quarantine period as well as at the time of necropsy under the direction of a qualified pathologist, physician or veterinarian having experience with diseases of rabbits, for the presence of signs or symptoms of ill health, particularly for evidence of tuberculosis, myxomatosis, fibromatosis, rabbit pox, and other diseases indigenous to rabbits. If there are any such signs, symptoms or other significant pathological lesions observed, tissues from that colony shall not be used in the production of vaccine.

(3) *Control vessels.* Control vessels shall be prepared, observed and tested as prescribed in §630.32(f).

(e) *Passage of virus strain in vaccine manufacture.* Virus in the final vaccine shall represent no more than five cell culture passages beyond the passage used as the seed strain for the manufacture of the vaccine used to perform the field studies (§630.60(d)), which qualified the manufacturer's vaccine strain for license.

(f) *Cell cultures in vaccine production areas.* Only the cell cultures used in the propagation of rubella virus vaccine shall be introduced into rubella virus vaccine production areas.

(g) *Test samples.* Test samples of rubella virus harvests or pools shall be withdrawn and maintained by following the procedures prescribed in §630.32(g).

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 47 FR 24699, June 8, 1982; 50 FR 4138, Jan. 29, 1985; 55 FR 47876, Nov. 16, 1990]

§630.63 Reference virus.

A Reference Rubella Virus, Live, shall be obtained from the Center for Biologics Evaluation and Research as a control for correlation of virus titers.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 630.64 Potency test.

The concentration of live rubella virus shall constitute the measure of potency. The titration shall be performed in a suitable cell culture system, using either the Reference Rubella Virus, Live, or a calibrated equivalent strain as a titration control. The concentration of live rubella virus contained in the vaccine of each lot under test shall be no less than the equivalent of 1,000 $TCID_{50}$ of the reference virus per human dose.

§ 630.65 Test for safety.

(a) *Tests prior to clarification of vaccine manufactured in duck embryo cell cultures.* Prior to clarification, the following tests shall be performed on each rubella virus pool prepared in duck embryo cell cultures:

(1) *Inoculation of adult mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(1), and the virus pool is satisfactory only if equivalent test results are obtained.

(2) *Inoculation of suckling mice.* The test shall be performed in the volume and following the procedures prescribed in § 630.35(a)(2), and the virus pool is satisfactory only if equivalent test results are obtained.

(3) *Inoculation of monkey tissue cell cultures.* A rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(4) *Inoculation of other cell cultures.* The rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in § 630.35(a)(3), in rhesus or cynomolgus monkey kidney, in chick embryo, duck embryo, and in human cell cultures except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if results equivalent to those in § 630.35(a)(3) are obtained.

(5) *Inoculation of embryonated chicken eggs.* A suspension of each undiluted rubella virus pool shall be tested in the volume and following the procedures prescribed in § 630.35(a)(5) except that the virus need not be neutralized by antiserum. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(6) *Inoculation of embryonated duck eggs.* A suspension of each undiluted rubella virus pool shall be tested in embryonated duck eggs, following the procedures prescribed in § 630.35(a)(5), except that the virus need not be neutralized by antiserum and the volume of inoculum per egg shall not exceed 1.0 milliliter. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(7) *Bacteriological tests.* In addition to the tests for sterility required pursuant to § 610.12 of this chapter, bacteriological tests shall be performed on each rubella virus pool for the presence of M. tuberculosis, both avian and human, by appropriate culture methods. The virus pool is satisfactory only if found negative for M. tuberculosis, both avian and human.

(8) *Test for avian leucosis.* The vaccine shall be tested for avian leucosis, in the volume and following the procedures prescribed in § 630.35(a)(8). The cultures are satisfactory for vaccine manufacture if found negative for avian leucosis.

(9) *Inoculation of cell cultures and embryonated eggs after neutralization of the virus with antiserum.* Each of the tests prescribed in paragraphs (a)(3), (4), (5), and (6) of this section shall be carried out also with rubella virus that has been neutralized by the addition of high titer antiserum of nonhuman, nonsimian and nonavian origin except that the volume of virus suspension of each undiluted virus pool tested shall be no less than 5 ml. The rubella antiserum shall have been prepared by using a rubella virus propagated in a cell culture system other than that used for the manufacture of the vaccine under test, and the cell culture system shall be free of extraneous agents which might elicit antibodies that could inhibit growth of any known extraneous agents which might be present in the vaccine under test. These tests may be performed either before or after clarification of the virus. The virus pool is satisfactory only if the results obtained are

equivalent to those required in those subparagraphs.

(b) [Reserved]

(c) *Tests prior to clarification of vaccine manufactured in rabbit renal cell cultures.* Prior to clarification each rubella virus pool prepared in rabbit renal cell cultures shall be tested as follows:

(1) *Inoculation of adult mice.* The test shall be performed in the volume and following the procedures prescribed in §630.35(a)(1), and the virus pool is satisfactory only if equivalent test results are obtained.

(2) *Inoculation of suckling mice.* The test shall be performed in the volume and following the procedures prescribed in §630.35(a)(2), and the virus pool is satisfactory only if equivalent test results are obtained.

(3) *Inoculation of monkey tissue cell cultures.* A rubella virus pool shall be tested for adventitious agents in the volume and following the procedures prescribed in §630.35(a)(3), except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(4) *Inoculation of other cell cultures.* The tests shall be performed in the volume and following the procedures prescribed in §630.35(a)(3) in rhesus or cynomolgus monkey kidney tissue, rabbit renal tissue and human tissue cell cultures, except that the virus need not be neutralized by antiserum. The rubella virus pool is satisfactory only if equivalent test results are obtained.

(5) *Inoculation of embryonated chicken eggs.* A suspension of each undiluted rubella virus pool shall be tested in the volume and following the procedures prescribed in §630.35(a)(5) except that the virus need not be neutralized by antiserum. The virus pool is satisfactory only if there is no evidence of adventitious agents.

(6) *Inoculation of rabbits.* A minimum of 15 ml. of each virus pool shall be tested by inoculation into at least five healthy rabbits, each weighing 1500–2500 grams. Each rabbit shall be injected intradermally in multiple sites with a total of 1.0 ml. and subcutaneously with 2.0 ml., of the virus pool, and the animals observed for at least 30 days. Each rabbit that dies after the first 24 hours of the test or is sacrificed because of illness shall be necropsied and the brain and organs removed and examined. The virus pool is satisfactory only if at least 80 percent of the rabbits remain healthy and survive the entire period and if all the rabbits used in the test fail to show lesions of any kind at the sites of inoculation and fail to show evidence of any viral infection.

(7) *Inoculation of guinea pigs.* Each of at least five guinea pigs, each weighing 350–450 grams, shall be inoculated intracerebrally with 0.1 ml. and intraperitoneally with 5 ml. of the undiluted virus pool. The animals shall be observed for at least 42 days. Each animal that dies after the first 24 hours of the test or is sacrificed because of illness, shall be necropsied. All remaining animals shall be sacrificed and necropsied at the end of the observation period. The virus pool is satisfactory only if at least 80 percent of all animals remain healthy and survive the observation period and if all the animals used in the test fail to show evidence of infection with *M. tuberculosis* or any viral infection.

(8) *Bacteriological tests.* In addition to the tests for sterility required pursuant to §610.12 of this chapter, bacteriological tests shall be performed on each rubella virus pool for the presence of *M. tuberculosis,* human, by appropriate culture methods. The rubella virus pool is satisfactory only if found negative for *M. tuberculosis,* human.

(9) *Tests for adventitious agents.* Each virus pool shall be tested for the presence of such known adventitious agents of rabbits as toxoplasma, encephalitozoon, herpes cuniculi, the vacuolating virus of rabbits, rabbit syncytial virus, myxoviruses and reoviruses. The virus pool is satisfactory only if the results of all tests show no evidence of any extraneous agent attributable to the rabbit renal tissue or the vaccine.

(10) *Inoculation of cell cultures and embryonated eggs after neutralization of the virus with antiserum.* Each of the tests prescribed in paragraphs (c)(3), (4), and (5) of this section shall be carried out also with rubella virus that has been neutralized by the addition of

high titer antiserum of nonhuman, nonsimian and nonrabbit origin following the procedures and in the volume prescribed in paragraph (a)(9) of this section. The virus pool is satisfactory only if the results obtained are equivalent to those required by that paragraph.

(d) *Clarification.* The rubella virus fluids shall be clarified by following the procedures prescribed in § 630.35(c).

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 40 FR 25813, June 19, 1975]

§ 630.66 General requirements.

(a) *Final container tests.* In addition to the tests required pursuant to § 610.14 of this chapter, an immunological and virological identity test shall be performed on the final container if it was not performed on each pool or on the bulk vaccine prior to filling.

(b) *Dose.* These standards are based on an individual human immunizing dose of no less than 1,000 $TCID_{50}$ of Rubella Virus Vaccine Live, expressed in terms of the assigned titer of the Reference Rubella Virus, Live.

(c) *Labeling.* In addition to the items required by other applicable labeling provisions of this subchapter, single dose container labeling for vaccine which is not protected against photochemical deterioration shall include a statement cautioning against exposure to light.

(d) *Photochemical deterioration; protection.* Rubella Virus Vaccine Live, in multiple dose containers, shall be protected against photochemical deterioration in accordance with the procedures prescribed in § 630.36(g).

(e) *Samples; protocols; offical release.* The following shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892:

(1) For each lot of vaccine:

(i) A protocol, which consists of a summary of the history of the manufacture of the lot, including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.

(ii) A total of no less than two 25-milliliter volumes, in a frozen state (−60° C.), of preclarification bulk vaccine containing no preservative or adjuvant.

(iii) A total of no less than 30 containers of the vaccine from each filling of each bulk lot of single-dose containers. A total of no less than six 50-dose containers or ten 10-dose containers of the vaccine from each filling of each bulk lot of multiple-dose containers.

(2) In addition to the requirements of paragraph (e)(1) of this section, whenever a new production seed lot is introduced, or whenever the source of cell culture substrate must be reestablished and recertified, samples consisting of no less than 100 milliliters in 10-milliliter volumes, in a frozen state (−60° C.), of postclarification bulk vaccine containing stabilizer but no preservative or adjuvant, taken from each of 5 consecutive lots of the bulk vaccine.

(3) The product shall not be issued by the manufacturer until written notification of official release of the lot is received from the Director, Center for Biologics Evaluation and Research.

[38 FR 32068, Nov. 20, 1973, as amended at 41 FR 10430, Mar. 11, 1976; 42 FR 27582, May 31, 1977; 49 FR 23834, June 8, 1984; 50 FR 4138, Jan. 29, 1985; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

Subpart H—Smallpox Vaccine

§ 630.70 Smallpox Vaccine.

(a) *Proper name and definition.* The proper name of this product shall be Smallpox Vaccine, which shall be a preparation of live vaccinia virus obtained from inoculated calves or chicken embryos.

(b) *Strains of virus.* The strain of seed virus used in the manufacture of Smallpox Vaccine shall be identified by historical records including origin and manipulation, and shall meet the sterility test requirements when tested by the procedure prescribed in § 610.12 of this chapter. The strain of seed virus and every third passage shall be tested by a rabbit scarification procedure and shown to maintain its original dermatropic properties. The test procedure is available upon request from the Director, Center for Biologics Evaluation and Research. Any new strain shall be shown not to produce a

reactivity in man exceeding that produced by the Reference Smallpox Vaccine.

[38 FR 32068, Nov. 20, 1973, as amended at 41 FR 51010, Nov. 19, 1976; 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 630.71 Production.

Vaccinia virus used for the manufacture of vaccine shall be obtained from vesicles on the skin of an inoculated calf or from inoculated chorioallantoic membranes of chicken embryos, as set forth below:

(a) *Virus from calves*—(1) *Quarantine.* Only calves which, prior to being placed in quarantine have reacted negatively to tuberculin, were afebrile and free of ectoparasites, and which shall have met all other applicable quarantine requirements of § 600.11(f)(2)(i) of this chapter, shall be used for vaccinia virus production. The quarantine period shall be at least 14 days. During the last 7 days of the quarantine period daily morning and afternoon rectal temperatures shall be taken and calves that do not remain afebrile during that period shall not be used for virus production.

(2) *Inoculation.* A larger area of the calf than will be used for production purposes shall be prepared in a manner comparable to that appropriate for aseptic surgery, except that the area to be inoculated must be washed free of all antiseptics that may have a deleterious effect on virus propagation. The instrument and method used for scarification must produce a uniform penetration into the epidermis but must not extend through into the corium.

(3) *Incubation.* The inoculated calf shall remain in the incubation room confined to its stall and daily morning and afternoon rectal temperatures shall be taken to determine that only the expected febrile condition occurs. If any signs of disease other than vesiculation at the inoculation site occur, the virus from that calf shall not be used for vaccine manufacture.

(4) *Harvesting.* Before harvesting, the calf shall be anesthetized and killed by exsanguination. Prior to harvesting, the inoculated area shall be thoroughly cleansed by aseptic techniques. Only the vesicular material shall be harvested.

(5) *Necropsy.* A necropsy shall be made of each production calf. The harvested material shall not be used from any animal suspected of having an infection other than vaccinia.

(b) *Virus from embryonated chicken eggs*—(1) *Eggs for production.* Embryonated chicken eggs used for propagation of vaccinia virus shall be derived from flocks found to be free of, and continuously monitored for freedom from *Salmonella pullorum,* Mycoplasma species, avian tuberculosis, fowl pox, Newcastle disease virus, Rous sarcoma virus, avian leucosis complex of viruses, and other agents pathogenic for chickens, or appropriate tests shall be performed to demonstrate freedom of the vaccine from such agents.

(2) *Harvesting.* Aseptic techniques shall be used in harvesting the chorioallantoic membranes exhibiting vesicles characteristic of vaccinia infection.

§ 630.72 Reference vaccine.

Reference Smallpox Vaccine and reconstitution fluid shall be obtained from the Center for Biologics Evaluation and Research and shall be used in all tests for determining the potency of Smallpox Vaccine.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 630.73 Potency test.

Each filling of Smallpox Vaccine shall be tested for potency by the ''pock count'' method as follows:

(a) [Reserved]

(b) *Pock counting in embryonated chicken eggs*—(1) *Dilutions* shall be made starting with no less than 0.5 ml. of the test vaccine and of the reference vaccine. The same diluent shall be used for all dilutions of both vaccines. The sample of vaccine in capillary tubes shall be obtained by pooling the contents of no less than 50 capillaries into a sterile vessel.

(2) *Inoculation of embryonated chicken eggs.* One-tenth milliliter of each dilution of test vaccine shall be inoculated onto the chorioallantoic membrane of each of at least five embryonated chicken eggs. The reference vaccine shall be tested in the same manner.

After inoculation, all eggs shall be incubated at 37°C±1°C for 48 hours.

(3) *Estimation of potency.* Only membranes from living embryos shall be removed and the number of specific lesions thereon shall be counted and recorded. The number of pock forming units in 1.0 ml. of vaccine shall be calculated from the number of lesions, the dilution factor and the volume used, to determine the titer of the undiluted vaccine. The accuracy of the titration shall be confirmed in each test by performing simultaneously the same type of titration with the reference vaccine which shall demonstrate its assigned titer.

(4) *Potency requirements*—(i) *Vaccine intended for multiple pressure administration.* Vaccine intended for multiple pressure administration shall have a titer at least equivalent to the reference vaccine.

(ii) *Vaccine intended for jet injection.* Vaccine intended for administration by jet injector shall have a number of pock forming units in one human dose at least equivalent to that contained in 0.1 ml. of the reference vaccine diluted 1:30.

(iii) *Heated liquid vaccine.* Samples of liquid vaccine from final containers taken at random shall be incubated at 35° to 37° C. for at least 18 hours, after which the heated sample shall be tested in parallel with a sample of unheated vaccine of the same lot, as prescribed in this paragraph. The vaccine is satisfactory if the heated sample retains at least one tenth of the potency of the unheated sample.

(iv) *Heated dried vaccine.* Samples of dried vaccine from final containers taken at random shall be incubated at 35° to 37° C. for 30 days, after which the heated sample shall be tested in parallel with a sample of unheated vaccine of the same lot, as prescribed in this paragraph. The vaccine is satisfactory if the heated sample retains at least one-tenth of the potency of the unheated sample.

[38 FR 32068, Nov. 20, 1973, as amended at 41 FR 51010, Nov. 19, 1976]

§ 630.74 Tests for safety.

(a) *Anaerobes.* A 10-milliliter sample representative of the homogenized viral harvest or pool of several viral harvests shall be tested for the presence of anaerobes in the following manner: Before the addition of preservatives other than glycerin, the test sample shall be inoculated into freshly heated Fluid Thioglycollate Medium using a ratio of inoculum to culture medium sufficient for optimal bacterial growth. The test vessels shall be incubated at 35° to 37° C and observed daily for 10 days for evidence of bacterial growth. If bacterial growth is observed, the organism(s) shall be identified as to genus. Within 24 to 48 hours of an indication that there may be anaerobic growth, 1.0-milliliter samples from each vessel showing growth shall be inoculated subcutaneously into each of at least three mice weighing not more than 20 grams each, and into each of three guinea pigs weighing not more than 350 grams each. The animals shall be observed daily for 6 days for signs of tetanus or presence of other anaerobes. If the animals show no signs of tetanus or presence of other anaerobes, additional groups of the same types and numbers of animals shall be injected 9 days after evidence of anaerobic bacterial growth is observed in the original planting with 1.0-milliliter samples from each test vessel showing growth. The animals shall be observed daily for 6 days for signs of tetanus or presence of other anaerobes. If any animals die within 3 days without having shown signs of tetanus or presence of other anaerobes, the test shall be repeated within 18 hours of the deaths, with 0.1-milliliter samples of the culture from which that animal was inoculated. Samples from the culture shall be injected into each of three additional test animals of the same species, and the animals shall be observed daily for 6 days. If there is any evidence of the presence of pathogenic anaerobes, the viral harvest may not be used in the manufacture of Smallpox Vaccine.

(b) [Reserved]

(c) *Coliform organisms.* A 5.0 ml. sample of bulk vaccine shall be tested for the presence of coliform organisms by the method published by the American Public Health Association, Inc., in ''Standard Methods for the Examination of Water and Wastewater'' (13th edition, 1971), section entitled ''Multiple-Tube Fermentation Technic for

Members of the Coliform Group,'' pages 662–678 and any amendments or revisions thereof, which section is hereby incorporated by reference and deemed published herein. Said publication is available at most medical and public libraries and copies of the pertinent section will be provided to any manufacturer affected by the provisions of this part upon request to the Director, Center for Biologics Evaluation and Research, or to the appropriate Information Center Officer listed in 45 CFR part 5. In addition, an official historic file of the material incorporated by reference is maintained in the Office of the Director, Center for Biologics Evaluation and Research, or available for inspection at the Office of the Federal Register, 800 North Capitol Street NW., suite 700, Washington, DC 20408. A method different than that contained in the above cited section may be used to test for the presence of coliform organisms upon a showing that it is of equal or greater sensitivity. The ratio of the volume of inoculum to the volume of culture medium shall be such as will dilute the preservative to a level that does not inhibit growth of contaminating organisms. The vaccine is satisfactory if there is no evidence of coliform organisms.

(d) *Hemolytic streptococci and coagulase-positive staphylococci.* Each of three 1.0 ml. samples of bulk vaccine shall be spread uniformly on the surface of separate blood agar plates. The plates shall be incubated for 48 hours at 35° to 37° C. The vaccine is satisfactory if there is no evidence of the presence of either hemolytic streptococci or coagulase-positive staphylococci.

(e) *Viable bacteria*—(1) *Vaccine intended for multiple pressure administration.* Samples of each lot of both bulk and final container vaccine shall be tested for viable bacteria by a procedure designed to detect both aerobic and anaerobic growth through a period of 7 days. At least three 1.0 ml. samples of bulk vaccine and three 0.2 ml. samples of vaccine derived from not less than three final containers or dilutions thereof shall be inoculated into a volume of culture medium sufficient for optimal bacterial growth. The vaccine is satisfactory if it contains no more than 200 viable organisms per ml.

(2) *Vaccine intended for jet injection.* Samples of each lot of both bulk and final container vaccine shall be tested for viable bacteria in Fluid Thioglycollate Medium prepared in accordance with §610.12(e)(1)(i) of this chapter for at least a 7-day test period. A sample of at least 10.0 ml. of bulk vaccine and 1.0 ml. from each of at least 20 final containers shall be tested. The ratio of the volume of the inoculum to the volume of culture medium shall be such as will dilute the preservative in the inoculum to a level that does not inhibit growth of contaminating micro-organisms. The vaccine is satisfactory if it contains no more than one organism per 100 doses of vaccine.

(f) *Sterile vaccine.* The tests prescribed in paragraphs (c), (d), and (e) of this section need not be performed on a lot of Smallpox Vaccine that meets the sterility requirements prescribed in §610.12 of this chapter.

[38 FR 32068, Nov. 20, 1973, as amended at 41 FR 51010, Nov. 19, 1976; 47 FR 9397, Mar. 5, 1982; 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§630.75 General requirements.

(a) *General safety.* Each lot of vaccine shall be tested for safety as prescribed in §610.11 of this chapter and shall meet the safety requirements of that section, except that for liquid Smallpox Vaccine distributed in capillaries, the test may be performed with a sample of bulk vaccine taken at the time of filling into final containers.

(b) *Preservative.* A preservative that meets the requirements of §610.15 of this chapter may be used, provided that if the preservative is phenol, its concentration shall not exceed 0.5 percent.

(c) *Labeling.* In addition to complying with all other applicable labeling provisions of this subchapter the package label shall bear the following:

(1) *Vaccine intended for jet injection.* (i) A conspicuous statement that the vaccine is intended for administration by jet injector.

(ii) A statement that the vaccine has been shown by appropriate test methods to contain not more than one organism per 100 doses or reference to an enclosed circular that contains such

information, except that such a statement is not required for vaccine which meets the sterility requirements of §610.12 of this chapter.

(2) *Vaccine intended for multiple pressure administration.* A statement that the vaccine has been shown by appropriate test methods to contain not more than 200 organisms per ml. or reference to an enclosed circular that contains such information, except that such a statement is not required for vaccine which meets the sterility requirements of §610.12 of this chapter.

(d) *Samples; protocols; official release.* (1) For each lot of vaccine the following shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892:

(i) A protocol which consists of a summary of the history of manufacture of each filling including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.

(ii) Three hundred capillaries from the first filling of a lot of liquid vaccine, and 200 capillaries from each subsequent filling.

(iii) Two 10 ml. samples of bulk liquid vaccine to be submitted along with the capillaries from the first filling and taken from the same vessel from which such capillaries were filled.

(iv) For vaccine intended for jet gun injection, a sample from each drying consisting of no less than eight 100-dose vials or eight 500-dose vials of vaccine in final labeled containers, plus sufficient diluent in final labeled containers to reconstitute the vaccine.

(v) For vaccine intended for multiple pressure administration, a sample from each drying consisting of no less than eighty 10-dose vials, ninety 25-dose vials, or eighty 100-dose vials of vaccine in final labeled containers, plus sufficient diluent in final labeled containers to reconstitute the vaccine.

(2) The product shall not be issued by the manufacturer until written notification of official release of the lot is received from the Director, Center for Biologics Evaluation and Research.

[38 FR 32068, Nov. 20, 1973, as amended at 42 FR 27582, May 31, 1977; 42 FR 56112, Oct. 21, 1977; 49 FR 23834, June 8, 1984; 51 FR 15610, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

PART 640—ADDITIONAL STANDARDS FOR HUMAN BLOOD AND BLOOD PRODUCTS

Subpart A—Whole Blood

Sec.

Subpart B—Red Blood Cells

Subpart C—Platelets

Subpart D—Plasma

Subpart E—[Reserved]

Subpart F—Cryoprecipitate